```
ILIGHT set on as ' '
? b 155, 5
       29dec02 10:41:30 User242957 Session D561.2
            $0.00    0.071 DialUnits File410
     $0.00  Estimated cost File410
     $0.03  TELNET
     $0.03  Estimated cost this search
     $0.03  Estimated total session cost   0.232 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Nov W4
*File 155: For updating information please see Help News155. Alert
feature enhanced with customized scheduling. See HELP ALERT.
  File   5:Biosis Previews(R)  1969-2002/Dec W4
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? s secondary and structure and rna
          435931  SECONDARY
          934965  STRUCTURE
          714059  RNA
      S1   10838  SECONDARY AND STRUCTURE AND RNA
? s s1 and polymerase
           10838  S1
          374087  POLYMERASE
      S2    1615  S1 AND POLYMERASE
? s s2 and reduc?
            1615  S2
         2028825  REDUC?
      S3     163  S2 AND REDUC?
? rd
...examined 50 records  (50)
...examined 50 records  (100)
...examined 50 records  (150)
...completed examining records
      S4     124  RD (unique items)
? s s4 and analog
             124  S4
           83981  ANALOG
      S5       0  S4 AND ANALOG
? t s4/3,ab/all

 4/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13754929   22199352   PMID: 12208995
  Signal  transduction and regulatory mechanisms involved in control of the
sigma(S) (RpoS) subunit of RNA polymerase.
  Hengge-Aronis Regine
  Institut fur Biologie, Mikrobiologie, Freie Universitat Berlin, 14195
Berlin, Germany. Rhenggea@zedat.fu-berlin.de
  Microbiology and molecular biology reviews : MMBR (United States)  Sep
2002, 66 (3) p373-95, table of contents, ISSN 1092-2172
Journal Code: 9706653
  Document type: Journal Article; Review; Review, Tutorial
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The sigma(S) (RpoS) subunit of RNA polymerase is the master
regulator of the general stress response in Escherichia coli and related
bacteria. While rapidly growing cells contain very little sigma(S),
```

July 20, 1999

202

```
 b 155, 5
       29dec02 10:58:55 User242957 Session D562.2
            $0.00    0.139 DialUnits File410
     $0.00  Estimated cost File410
     $0.43  TELNET
     $0.43  Estimated cost this search
     $0.43  Estimated total session cost   0.286 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Nov W4
*File 155: For updating information please see Help News155. Alert
feature enhanced with customized scheduling. See HELP ALERT.
  File   5:Biosis Previews(R)  1969-2002/Dec W4
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? s analogue
      S1   80109  ANALOGUE
? s s1 and rna and polymerase and t7
           80109  S1
          714059  RNA
          374087  POLYMERASE
           11935  T7
      S2      38  S1 AND RNA AND POLYMERASE AND T7
? rd
...completed examining records
      S3      25  RD (unique items)
? s s3 and py<2000
              25  S3
        22894648  PY<2000
      S4      21  S3 AND PY<2000
? t s4/3,ab/all

 4/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10296467   99263030   PMID: 10325415
  The environment of 5S rRNA in the ribosome: cross-links to 23S rRNA from
sites within helices II and III of the 5S molecule.
  Osswald M; Brimacombe R
  Max-Planck-Institut fur Molekulare Genetik, Ihnestrasse 73, 14195 Berlin,
Germany.
  Nucleic acids research (ENGLAND)   Jun 1 1999,  27  (11)  p2283-90,
ISSN 0305-1048   Journal Code: 0411011
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Three contiguous fragments of Escherichia coli 5S rRNA were prepared by
T7 transcription from synthetic DNA templates. The central fragment,
comprising residues 33-71 of the molecule, was transcribed in the presence
of 4-thiouridine triphosphate together with [32P]UTP. The three transcripts
were ligated together, yielding a 5S rRNA analogue carrying
4-thiouridine residues at positions 40, 48, 55 and 65 in helices II and
III. After ligation, the 4-thiouridine residues were derivatised with p
-azidophenacyl bromide. The modified 5S rRNA was reconstituted into 50S
subunits and these subunits were used to prepare 70S ribosomes in the
presence or absence of tRNA and mRNA. The azidophenyl groups were then
photoactivated by mild irradiation at 300 nm and the products of
cross-linking analysed by our standard procedures. Multiple cross-links
from 5S rRNA to two distinct regions of the 23S rRNA were observed. The
```

first region was located in helix 38 in Domain II of the 23S molecule, with cross-links at sites between nucleotides 885 and 922. The second region covered helices 81-85 in Domain V, with sites between nucleotides 2272 and 2345. Taken together with previous data, these results serve to define the arrangement of the 5S rRNA molecule relative to the 23S rRNA within the 50S subunit.

4/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10256441 99252211 PMID: 10233981
A single amino acid substitution in the phosphoprotein of respiratory syncytial virus confers thermosensitivity in a reconstituted **RNA polymerase** system.
Marriott A C; Wilson S D; Randhawa J S; Easton A J
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom.
Journal of virology (UNITED STATES) Jun **1999**, 73 (6) p5162-5, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The single amino acid change Gly172 to Ser in the phosphoprotein (P) of respiratory syncytial virus (RSV) has previously been shown to be responsible for the thermosensitivity and protein-negative phenotype of tsN19, a mutant of the B subgroup RSN-2 strain. This single change was inserted into the P gene of the A subgroup virus RSS-2, and the resulting phenotype was observed in a plasmid-driven reconstituted RSV **RNA polymerase** system. Expression from a genome **analogue** containing two reporter genes was thermosensitive when directed by plasmids containing the N, L, M2, and mutant P genes cloned under the control of **T7** promoters. Analysis of **RNA** synthesis showed that mutant P protein was unable to produce genome, antigenome, or mRNA at the restrictive temperature. At a semipermissive temperature, genome, antigenome, and mRNA synthesis were all reduced, 6- to 30-fold, relative to synthesis directed by a wild-type P plasmid. Binding of the mutant P protein to N protein in the absence of other viral proteins was unaffected by temperature, indicating that the lesion did not produce a large enough structural change to disrupt this binding. These data suggest that the plasmid rescue system is suitable for investigation of the role of thermosensitive mutations in RSV **polymerase** components in **RNA** synthesis.

4/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09979611 98409449 PMID: 9737873
Identifying **RNA** minor groove tertiary contacts by nucleotide **analogue** interference mapping with N2-methylguanosine.
Ortoleva-Donnelly L; Kronman M; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
Biochemistry (UNITED STATES) Sep 15 **1998**, 37 (37) p12933-42, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Nucleotide **analogue** interference mapping (NAIM) is a general biochemical method that rapidly identifies the chemical groups important for **RNA** function. In principle, NAIM can be extended to any nucleotide that can be incorporated into an in vitro transcript by an

RNA polymerase . Here we report the synthesis of 5'-O-(1-thio)-N2-methylguanosine triphosphate (m2GalphaS) and its incorporation into two reverse splicing forms of the Tetrahymena group I intron using a mutant form of **T7 RNA polymerase**. This **analogue** replaces one proton of the N2 exocyclic amine with a methyl group, but is as stable as guanosine (G) for secondary structure formation. We have identified three sites of m2GalphaS interference within the Tetrahymena intron: G22, G212, and G303. All three of these guanosine residues are known to utilize their exocyclic amino groups to participate in tertiary hydrogen bonds within the ribozyme structure. Unlike the interference pattern with the phosphorothioate of inosine (IalphaS, an **analogue** that deletes the N2 amine of G), m2GalphaS substitution did not cause interference at positions attributable to secondary structural stability effects. Given that the **RNA** minor groove is likely to be widely used for helix packing, m2GalphaS provides an especially valuable reagent to identify **RNA** minor groove tertiary contacts in less well-characterized RNAs.

4/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09975416 98418505 PMID: 9747733
Sequence analysis of a functional **polymerase** (L) gene of bovine respiratory syncytial virus: determination of minimal trans-acting requirements for **RNA** replication.
Yunus A S; Collins P L; Samal S K
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park 20742, USA.
Journal of general virology (ENGLAND) Sep **1998**, 79 (Pt 9) p2231-8, ISSN 0022-1317 Journal Code: 0077340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The complete nucleotide sequence of a functional clone of the large **polymerase** (L) gene of bovine respiratory syncytial virus (BRSV) strain A51908 was determined by analysis of cloned cDNAs obtained from genomic and mRNAs. The BRSV L gene is 6573 nt in length and the derived polypeptide has 2162 aa. Alignment of the sequences of the BRSV L gene, and its encoded protein, with sequences of the L gene and protein of human respiratory syncytial virus strain A2 showed 77% identity at the nucleotide level and 84% identity at the amino acid level. By comparison, the L gene and protein of avian pneumovirus showed only 50% identity at the nucleotide level and 64% identity at the amino acid level. A minigenome was constructed to encode a BRSV vRNA **analogue** containing the gene for chloramphenicol acetyltransferase (CAT) under the control of putative BRSV transcription motifs and flanked by the BRSV genomic termini. Transfection of plasmids encoding the BRSV minigenome, nucleocapsid protein (N), phosphoprotein (P) and L protein, each under the control of **T7** promoter, into cells infected with a vaccinia virus recombinant expressing the **T7 RNA polymerase** gave rise to CAT activity and progeny with the minigenome. This result indicates that the N, P and L proteins are necessary and sufficient for transcription and replication of the BRSV minigenome and are functional. Further, inclusion of small amounts of the M2 protein along with the N, P and L proteins greatly augmented minigenome transcription.

4/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09799714 98241249 PMID: 9582093
The chemical basis of adenosine conservation throughout the Tetrahymena

ribozyme.
Ortoleva-Donnelly L; Szewczak A A; Gutell R R; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
RNA (New York, N.Y.) (UNITED STATES) May **1998**, 4 (5) p498-519, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM48207; GM; NIGMS; GM54839; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adenosines are present at a disproportionately high frequency within several **RNA** structural motifs. To explore the importance of individual adenosine functional groups for group I intron activity, we performed Nucleotide Analog Interference Mapping (NAIM) with a collection of adenosine analogues. This paper reports the synthesis, transcriptional incorporation, and the observed interference pattern throughout the Tetrahymena group I intron for eight adenosine derivatives tagged with an alpha-phosphorothioate linkage for use in NAIM. All of the analogues were accurately incorporated into the transcript as an A. The sites that interfere with the 3'-exon ligation reaction of the Tetrahymena intron are coincident with the sites of phylogenetic conservation, yet the interference patterns for each **analogue** are different. These interference data provide several biochemical constraints that improve our understanding of the Tetrahymena ribozyme structure. For example, the data support an essential A-platform within the J6/6a region, major groove packing of the P3 and P7 helices, minor groove packing of the P3 and J4/5 helices, and an axial model for binding of the guanosine cofactor. The data also identify several essential functional groups within a highly conserved single-stranded region in the core of the intron (J8/7). At four sites in the intron, interference was observed with 2'-fluoro A, but not with 2'-deoxy A. Based upon comparison with the P4-P6 crystal structure, this may provide a biochemical signature for nucleotide positions where the ribose sugar adopts an essential C2'-endo conformation. In other cases where there is interference with 2'-deoxy A, the presence or absence of 2'-fluoro A interference helps to establish whether the 2'-OH acts as a hydrogen bond donor or acceptor. Mapping of the Tetrahymena intron establishes a basis set of information that will allow these reagents to be used with confidence in systems that are less well understood.

4/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09787825 98213737 PMID: 9547267
Synthesis and **RNA polymerase** incorporation of the degenerate ribonucleotide **analogue** rPTP.
Moriyama K; Negishi K; Briggs M S; Smith C L; Hill F; Churcher M J; Brown D M; Loakes D
Gene Research Centre, Okayama University, Tsushima, Okayama 700, Japan, Nycomed Amersham plc, Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire HP7 9LL, UK.
Nucleic acids research (ENGLAND) May 1 **1998**, 26 (9) p2105-11, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The synthesis and enzymatic incorporation into **RNA** of the hydrogen bond degenerate nucleoside **analogue** 6-(beta-d-ribofuranosyl)-3, 4-dihydro-8H-pyrimido[4,5-c]-[1,2]oxazin-7-one (P) is described. The 5'-triphosphate of this **analogue** is readily incorporated by T3, **T7** and SP6 **RNA** polymerases into **RNA** transcripts, being best incorporated in place of UTP, but also in place of CTP. When all the

uridine residues in an HIV-1 TAR **RNA** transcript are replaced by P the transcript has similar characteristics to the wild-type TAR **RNA**, as demonstrated by similar melting temperatures and CD spectra. The P-substituted TAR transcript binds to the Tat peptide ADP-1 with only 4-fold lowered efficiency compared with wild-type TAR.

4/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09740162 98169155 PMID: 9510335
Enzymatic incorporation of 2'-thio-CTP into the HDV ribozyme.
Raines K; Gottlieb P A
Department of Biological Science, SUNY at Buffalo, New York 14260, USA.
RNA (New York, N.Y.) (UNITED STATES) Mar **1998**, 4 (3) p340-5,
ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM52033; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have synthesized the **analogue** 2'-deoxy-2'-thio-CTP (CTP-SH) and tested its ability to support **RNA** transcription in place of CTP. The modified nucleotide in a transcription reaction and in the absence of CTP generated the appropriately sized fragment when a mutant **T7 polymerase** (Y639F) was used. Wild-type **polymerase** was unable to generate **RNA** under the same conditions. Transcription was optimal around pH 7.5 and was dependent upon CTP-SH concentration. Transcripts containing the **analogue** were efficiently isolated using a thiol-activated sepharose column. Insertion of CTP-SH into the HDV ribozyme, replacing all cytidine residues with 2'-thiocytidine, appears to inhibit self-cleaving activity, even in the presence of manganese. The ability to introduce the CTP-SH **analogue** enzymatically into **RNA** opens the way for new structure-function studies where the 2'-hydroxyl can be efficiently replaced by a thiol group.

4/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09534383 97436528 PMID: 9292501
Photoaffinity labeling of 30S-subunit proteins S7 and S11 by 4-thiouridine-substituted tRNA(Phe) situated at the P site of Escherichia coli ribosomes.
Rosen K V; Zimmerman R A
Department of Biochemistry & Molecular Biology, University of Massachusetts, Amherst 01003-4505, USA.
RNA (New York, N.Y.) (UNITED STATES) Sep **1997**, 3 (9) p1028-36,
ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM22807; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
4-Thiouridine, a photoreactive **analogue** of uridine, was randomly incorporated into yeast tRNA(Phe) precursor molecules by transcription with **T7 RNA polymerase** and the resulting transcripts were converted into mature tRNA(Phe) by treatment with RNase P **RNA**. The photoreactive tRNA(Phe) was aminoacylated and bound to the P site of Escherichia coli 70S ribosomes in the presence of a poly(U) template. Irradiation of the complexes with light of 300 nm resulted in the covalent crosslinking of nt U20 in the D loop of the tRNA to protein S11 of the 30S ribosomal subunit, whereas nt U33 in the anticodon loop crosslinked to 30S-subunit protein S7. These results allowed us to map the D loop of P

site-bound tRNA to the platform of the 30S ribosomal subunit and provided additional information about contacts between protein S7 and the anticodon loop in the cleft between the platform and the subunit head.

4/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09497747 97392806 PMID: 9245591
Bioincorporation of telluromethionine into proteins: a promising new approach for X-ray structure analysis of proteins.
Budisa N; Karnbrock W; Steinbacher S; Humm A; Prade L; Neuefeind T; Moroder L; Huber R
Max-Planck Institut fur Biochemie, Martinsried, Germany.
Journal of molecular biology (ENGLAND) Jul 25 **1997**, 270 (4) p616-23, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A simple and efficient method for the specific and quantitative replacement of the naturally occurring amino acid methionine by its isosteric **analogue** telluromethionine in the expression of recombinant proteins has been developed. The method requires a controlable and competitive expression system like the bacteriophage **T7** **polymerase** /promoter in a methionine-auxotrophic host. Using methionine-auxotrophic Escherichia coli strains, incorporation of telluromethionine at high yields has been achieved for human recombinant annexin V, human mitochondrial transamidase, Arabidopsis glutathione-S-transferase and the N-terminal domain of Salmonella tailspike adhesion protein as confirmed by amino acid, mass-spectrometric and X-ray analyses. Expressed and purified telluromethionine-proteins and native proteins were found to crystallise isomorphously. In terms of efficient bio-expression, isomorphism of crystals and relative abundance of methionine residues, the production of telluromethionine-proteins as heavy-atom derivatives offers a valid and general approach in X-ray analysis by the method of multiple isomorphous replacement.

4/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09068615 96419174 PMID: 8821947
Purification of the Tn10-specified tetracycline efflux antiporter TetA in a native state as a polyhistidine fusion protein.
Aldema M L; McMurry L M; Walmsley A R; Levy S B
Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.
Molecular microbiology (ENGLAND) Jan **1996**, 19 (1) p187-95, ISSN 0950-382X Journal Code: 8712028
Contract/Grant No.: AI30646; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The bacterial tetracycline-resistance determinant from Tn10 encodes a 43 kDa membrane protein, TetA, responsible for active efflux of tetracyclines. The tetA gene was cloned behind a **T7** promoter/lac operator in a plasmid that provided fusion of TetA to a polyhistidine-carboxy terminal tail. A second plasmid provided a regulated **T7** **RNA** **polymerase** . The specific activity of the TetA fusion protein was between 10-40% that of the wild-type protein as assayed by tetracycline resistance in cells and by transport in membrane vesicles. The fusion protein, overproduced approximately 3-13-fold, was purified by nickel

chelation chromatography. Calculations from circular dichroism spectra of the purified protein solubilized in dodecylmaltoside gave an alpha-helix content of 54-64%, close to the 68% predicted from the amino acid sequence by hydropathy analysis (12 membrane-spanning helices) for the native protein in the membrane bilayer. Fluorescence studies showed binding activity of the purified protein to its substrate, the tetracycline **analogue** 13-(cyclopentylthio)-5-hydroxy-6-alpha-deoxytetracycline. These findings suggested that the purified protein was in a native state.

4/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08208287 94344242 PMID: 8065453
Interactions of a small **RNA** with antibiotic and **RNA** ligands of the 30S subunit.
Purohit P; Stern S
Program in Molecular Medicine, UMASS Medical Center, Worcester 01605.
Nature (ENGLAND) Aug 25 **1994**, 370 (6491) p659-62, ISSN 0028-0836 Journal Code: 0410462
Comment in Nature. 1994 Aug 25;370(6491) 597-8; Comment in PMID 8065445
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It is now generally accepted that 16S and 23S ribosomal **RNA** play important roles in the decoding and peptidyl transferase activities of ribosomes. Despite their complex structures and numerous associated proteins it is possible that small domains of these rRNAs can fold and function autonomously, particularly those that appear devoid of protein interactions. One candidate for such a domain is the decoding region, located near the 3' end of 16S rRNA (Fig. 1a, b). Consistent with this hypothesis, aminoglycoside antibiotics that interact with the decoding region in 30S subunits interact with other RNAs in the absence of proteins. In addition, certain activities of self-splicing introns, at least superficially, resemble translational decoding. We report here that an oligoribonucleotide **analogue** of the decoding region interacts with both antibiotic and **RNA** ligands of the 30S subunit in a manner that correlates with normal subunit function. The activities of the decoding region **analogue** suggest that the intimidating structural complexity of the ribosome can be, to some degree, circumvented.

4/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07671144 93194890 PMID: 8449951
Effects of DNA lesions on transcription elongation by **T7 RNA polymerase**.
Chen Y H; Bogenhagen D F
Department of Pharmacological Sciences, State University of New York, Stony Brook 11794-8651.
Journal of biological chemistry (UNITED STATES) Mar 15 **1993**, 268 (8) p5849-55, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: ES04068; ES; NIEHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
T7 phage **RNA polymerase** was used to transcribe a series of DNA templates bearing any of several precisely localized lesions. Lesions were positioned downstream of the **T7** promoter on either strand of the DNA template to investigate the effects of these lesions on elongation of transcription. The following four types of DNA modifications

were studied: 1) 3-hydroxy-2-hydroxymethyltetrahydrofuran (tetrahydrofuran), a synthetic apurinic/apyrimidinic site; 2) 8-oxoguanine (8-oxodG), an oxidized derivative of guanine; 3) N-acetyl-2-aminofluorene (AAF) modified guanine; 4) 2-aminofluorene (AF) modified guanine. None of these lesions blocked transcription elongation when they were located on the non-template strand. Lesions on the template strand blocked elongation with varied efficiency. The series of AAF-dG, AF-dG, and tetrahydrofuran lesions showed a progressively decreasing ability to block elongation, while 8-oxo-dG caused little, if any, premature termination. **T7 RNA polymerase** was able to read through all of the lesions with sufficient efficiency to permit chain termination sequencing using the read-through products as templates. AAF-dG and AF-dG adducts did not induce detectable misreading. Adenine and, more rarely, cytosine were incorporated opposite 8-oxo-dG, as observed for translesional synthesis by DNA polymerases. Adenine was most commonly inserted opposite the non-instructional abasic site **analogue**, although a minor fraction of guanine was incorporated.

4/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07476869 93003118 PMID: 1390741
2'-Fluoro- and 2'-amino-2'-deoxynucleoside 5'-triphosphates as substrates for **T7 RNA polymerase**.
Aurup H; Williams D M; Eckstein F
Max-Planck-Institut fur experimentelle Medizin, Gottingen, FRG.
Biochemistry (UNITED STATES) Oct 13 **1992**, 31 (40) p9636-41,
ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
2'-Fluoro- and 2'-amino-2'-deoxynucleoside 5'-triphosphates have been investigated as substrates for **T7 RNA polymerase** . Michaelis-Menten kinetic parameters are reported for the incorporation of 2'-fluoro-2'-deoxyuridine, 2'-fluoro-2'-deoxycytidine, and 2'-amino-2'-deoxyuridine into runoff transcripts. The 2'-amino derivative of uridine is a better substrate than the 2'-fluoro derivative. Gel electrophoretic analysis shows that full-length transcripts with a length of 2500 nucleotides can be obtained with the analogues, although a considerable amount of shorter fragments accompanies the full-length product. In keeping with the kinetic analysis, the 2'-aminouridine triphosphate gives a cleaner product than the 2'-fluoro **analogue**. Transcription of two tRNA genes shows that such shorter templates can be transcribed to full-length products essentially without premature termination with any of the analogues.

4/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07221661 92144548 PMID: 1737000
Binding of protein synthesis initiation factor 4E to oligoribonucleotides: effects of cap accessibility and secondary structure.
Carberry S E; Friedland D E; Rhoads R E; Goss D J
Department of Chemistry, Hunter College of the City University of New York 10021-5024.
Biochemistry (UNITED STATES) Feb 11 **1992**, 31 (5) p1427-32,
ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM 20818; GM; NIGMS; RR-0307; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
The binding of rabbit globin mRNA to the 25-kDa cap binding protein eIF-4E from human erythrocytes was found to be 5.3-fold stronger than the binding of the cap **analogue** m7GpppG to eIF-4E [Gross et al. (1990) Biochemistry 29, 5008-5012]. In order to investigate whether this effect is due to the longer sequence of nucleotides in globin mRNA or to other features such as cap accessibility or secondary structure, oligoribonucleotide analogues of rabbit alpha-globin mRNA were synthesized by **T7 RNA polymerase** from a synthetic oligodeoxynucleotide template in the presence of m7GpppG; these oligoribonucleotide analogues possess varying degrees of cap accessibility and secondary structure. Equilibrium association constants for the interaction of these oligoribonucleotides and purified human erythrocyte eIF-4E were obtained from direct fluorescence titration experiments. The data indicate that while the presence of the m7G cap is required for efficient recognition by eIF-4E, the cap need not be completely sterically accessible, since other structural features within the mRNA also influence binding.

4/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06803400 91105138 PMID: 1703019
Effects of 2-chloro-2'-deoxyadenosine 5'-triphosphate on DNA synthesis in vitro by purified bacterial and viral DNA polymerases.
Hentosh P; McCastlain J C; Blakley R L
Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.
Biochemistry (UNITED STATES) Jan 15 **1991**, 30 (2) p547-54, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: P30 CA 21765; CA; NCI; RO1 CA 39242; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
2-Chloro-2'-deoxyadenosine 5'-triphosphate (CldATP) was compared with dATP as a substrate for DNA synthesis by bacterial and viral DNA polymerases in vitro. Lengths of chain extension and DNA synthesis pause sites were determined by comparison with products generated by dideoxynucleotide sequencing methods on the same end-labeled primer/template duplex after high-resolution polyacrylamide gel electrophoresis. Reverse transcriptase (RT) from human immunodeficiency virus (HIV-1) and avian myeloblastosis virus (AMV) incorporated CldATP efficiently. DNA strand elongation continued past most chloroadenine (ClA) insertion sites but resulted in shorter chains than when dATP was inserted. Phage T4 DNA **polymerase** incorporated CldATP least efficiently; Klenow fragment of Escherichia coli DNA **polymerase** I and modified **T7** DNA **polymerase** (Sequenase) showed intermediate ability to utilize the **analogue**. Incorporation of several consecutive ClA residues into the replicating strand dramatically reduced the ability of Sequenase, Klenow fragment, and T4 DNA polymerases to continue strand elongation. In the absence of the corresponding normal deoxyribonucleoside triphosphate during DNA synthesis, ClA was frequently misincorporated as thymine, cytosine, or guanine by both AMV RT and HIV-1 RT but rarely, if at all, by Klenow fragment, Sequenase, and T4 DNA **polymerase**. Except T4, for most DNA polymerases, CldATP at 10-20-fold molar excess over dATP was not a strong competitive inhibitor of dATP, as judged by the amount of strand extension and **polymerase** pause sites during DNA synthetic reactions. Our results indicate that the degree of strand extension in the presence of CldATP, the number and location of **polymerase** pause sites, and the amount of misincorporation of the **analogue** are both **polymerase**- and sequence-dependent.

4/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06290434 89377826 PMID: 2776753
Biochemical and biophysical analysis of pseudoknot-containing **RNA** fragments. Melting studies and NMR spectroscopy.
van Belkum A; Wiersema P J; Joordens J; Pleij C; Hilbers C W; Bosch L
Department of Biochemistry, Gorlaeus Laboratories, Leiden, The Netherlands.
European journal of biochemistry / FEBS (GERMANY, WEST) Aug 15 **1989**, 183 (3) p591-601, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Three overlapping **RNA** fragments containing the pseudoknot, as found in the tRNA-like structure of turnip yellow mosaic virus (TYMV) **RNA**, have been isolated and purified. Site-directed cleavage of TYMV **RNA** by RNase H, followed by ammonium sulphate precipitation and ion-exchange HPLC, yielded a pure preparation of a 3'-terminal, 112-nucleotide TYMV **RNA** fragment. Transcription of TYMV cDNA by **T7 RNA polymerase** , resulted in the isolation of an 88-nucleotide fragment. Finally, a 44-nucleotide fragment containing the TYMV **RNA** pseudoknot and strongly resembling the aminoacyl acceptor arm of the viral **RNA** was also synthesised using **T7 RNA polymerase**. The three fragments were isolated in milligram amounts and used for biochemical structure mapping, ultraviolet melting studies and NMR spectroscopy. Chemical modification with diethyl pyrocarbonate and sodium bisulphite and enzymatic digestion with RNase T1 confirmed the presence of the pseudoknot in the 44-nucleotide fragment. Also the **analogue** of the T-stem and T-loop of the tRNA-like structure of TYMV **RNA** was found. The results of modification at various temperatures in Mg2+-containing buffers were in general agreement with optical melting studies. Ultraviolet melting analysis of the longer fragments revealed their greater complexity and the results appear similar to those obtained for some tRNA species. To obtain direct biophysical evidence for base-pairing and stacking interactions in the pseudoknot, NMR studies were initiated. The first proton-NMR spectra ever obtained for plant viral **RNA** fragments are presented. NMR spectra were recorded at various buffer conditions and at various temperatures. The spectra for the 112-nucleotide and 88-nucleotide fragment are too complicated to be solved at present. In the case of the 44-nucleotide fragment, however, the imino proton resonances are well separated and this system turns out to be most promising for structural studies.

4/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06219423 89302852 PMID: 2663058
T7 RNA polymerase does not interact with the 5'-phosphate of the initiating nucleotide.
Martin C T; Coleman J E
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510.
Biochemistry (UNITED STATES) Apr 4 **1989**, 28 (7) p2760-2, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM10902; GM; NIGMS; GM21919-13; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The study of transcription kinetics by **T7 RNA**

polymerase is facilitated by the small size of its promoter, allowing the use of synthetic oligonucleotide templates with carefully defined sequences. We have previously used this approach to measure Michaelis-Menten steady-state kinetics for production of the five-base runoff transcript GGACU. In particular, Km for the interaction between enzyme and template under saturating levels of all four nucleotide triphosphates was shown to be approximately 0.02 microM. We now show that the corresponding Km and Vmax for initiation on a similar template coding for the runoff transcript GACU are the same as for the earlier study (Km = 0.02 microM; kcat = 40-50 min-1). This new template allows the measurement Km for association of the initial nucleotide GTP with enzyme or with the enzyme-DNA complex. The results show that KGTPm (0.60 mM) is somewhat higher than earlier approximations of Km for addition of elongating GTP during the later phase of processive elongation. As expected, the (initiating) Km for the GTP **analogue** ITP (KITPm) is increased (by about 2-fold), presumably as a result of weakened Watson-Crick base pairing. However, comparison of Km values for the GTP analogues GMP and guanosine shows little effect on substitution of the 5'-triphosphate by monophosphate or by a hydroxyl, respectively. This result suggests that a single active site has been evolutionarily adapted to accept from the 5' end of a waiting nucleotide both a 5'-triphosphate at initiation and a 5'-monophosphate ester (**RNA**) during elongation.(ABSTRACT TRUNCATED AT 250 WORDS)

4/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06104772 89207489 PMID: 2853971
Binding interactions between yeast tRNA ligase and a precursor transfer ribonucleic acid containing two photoreactive uridine analogues.
Tanner N K; Hanna M M; Abelson J
Department of Biology, California Institute of Technology, Pasadena 91125.
Biochemistry (UNITED STATES) Nov 29 **1988**, 27 (24) p8852-61, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: 1 F32 GM11823; GM; NIGMS; GM 32637; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Yeast tRNA ligase, from Saccharomyces cerevisiae, is one of the protein components that is involved in the splicing reaction of intron-containing yeast precursor tRNAs. It is an unusual protein because it has three distinct catalytic activities. It functions as a polynucleotide kinase, as a cyclic phosphodiesterase, and as an **RNA** ligase. We have studied the binding interactions between ligase and precursor tRNAs containing two photoreactive uridine analogues, 4-thiouridine and 5-bromouridine. When irradiated with long ultraviolet light, **RNA** containing these analogues can form specific covalent bonds with associated proteins. In this paper, we show that 4-thiouridine triphosphate and 5-bromouridine triphosphate were readily incorporated into a precursor tRNA(Phe) that was synthesized, in vitro, with bacteriophage **T7 RNA polymerase**. The **analogue** -containing precursor tRNAs were authentic substrates for the two splicing enzymes that were tested (endonuclease and ligase), and they formed specific covalent bonds with ligase when they were irradiated with long-wavelength ultraviolet light. We have determined the position of three major cross-links and one minor cross-link on precursor tRNA(Phe) that were located within the intron and near the 3' splice site. On the basis of these data, we present a model for the in vivo splicing reaction of yeast precursor tRNAs.

4/3,AB/19 (Item 19 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

05478503 87230996 PMID: 2438652
Stereospecificity of nucleases towards phosphorothioate-substituted **RNA**: stereochemistry of transcription by **T7 RNA polymerase**.
Griffiths A D; Potter B V; Eperon I C
Nucleic acids research (ENGLAND) May 26 **1987**, 15 (10) p4145-62
, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Transcription by **T7 RNA polymerase** has been studied using a chiral ATP **analogue** . The Sp diastereoisomer of adenosine 5'-O-(1-thiotriphosphate) (ATP alpha S) was incorporated into **RNA** with an apparent KM of approximately 15 microM, similar to that for ATP; the Rp diastereoisomer was neither a substrate nor a competitive inhibitor. The configuration of the phosphodiester link in the **RNA** produced was analyzed with stereospecific nucleases. The rate of nuclease digestion was compared with the rate of digestion of phosphorothioate-substituted **RNA** of known stereochemistry synthesized by E. coli **RNA polymerase** . Surprisingly, the nucleases exhibited reduced discrimination compared with their activity on dinucleotides. The results show that phosphorothioate-substituted **RNA** transcribed by **T7 RNA polymerase** has the same configuration as that transcribed by E. coli **RNA polymerase**, ie. Rp. Thus, the reaction proceeds with inversion of configuration at phosphorus.

4/3,AB/20 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08991617 BIOSIS NO.: 199396143118
Preparation of infectious Venezuelan equine encephalitis virus based on full length DNA copy of its genome.
AUTHOR: Kolykhalov A A; Frolov I V; Agapov E V; Netesov S V; Sandakhchiev L S
AUTHOR ADDRESS: Res. Inst. Mol. Biol., Sci. Prod. Assoc. "Vector", Koltsovo, Novosibirsk**Russia
JOURNAL: Doklady Akademii Nauk 327 (1):p160-164 **1992**
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian; Non-English

ABSTRACT: An attempt was made to create an efficient experimental system to study the effect of mutation-induced changes in various genes on the biological properties of Venezuelan equine encephalitis (VEE) virus and first of all on its attenuation. Experiments were carried out with a Trinidad donkey strain of VEE virus. Plasmid pVE-57 constructed contained full-length DNA-copy of genome. The plasmid was then used for the synthesis of **RNA analogue** of VEE genome in in-vitro transcription reaction, using **RNA-polymerase** of phase **T7**. The results of the transfection of the cell culture by **RNA** preparations obtained under varying transcription conditions were presented. The biological properties of the VEE virus obtained on the basis of the genome copy and the initial strain were compared in mice. It was found that the viruses were identical. Molecular sequence data are presented.

1992

4/3,AB/21 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06595513 BIOSIS NO.: 000087037675
BINDING INTERACTIONS BETWEEN YEAST TRANSFER **RNA** LIGASE AND A PRECURSOR TRANSFER **RNA** CONTAINING TWO PHOTOREACTIVE URIDINE ANALOGUES
AUTHOR: TANNER N K; HANNA M M; ABELSON J
AUTHOR ADDRESS: DIV. BIOL., CALIF. INST. TECHNOL., PASADENA, CALIF. 91125.
JOURNAL: BIOCHEMISTRY 27 (24). 1988. 8852-8861. **1988**
FULL JOURNAL NAME: Biochemistry
CODEN: BICHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Yeast tRNA ligase, from Saccharomyces cerevisiae, is one of the protein components that is involved in the splicing reaction of intron-containing yeast precursor tRNAs. It is an unusual protein because it has three distinct catalytic activities. It functions as a polynucleotide kinase, as a cyclic phosphodiesterase, and as an **RNA** ligase. We have studied the binding interactions between ligase and precursor tRNAs containing two photoreactive uridine analogues, 4-thiouridine and 5-bromouridine. When irradiated with long ultraviolet light, **RNA** containing these analogues can form specific covalent bonds with associated proteins. In this paper, we show that 4-thiouridine triphosphate and 5-bromouridine triphosphate were readily incorporated into a precursor tRNAPhe that was synthesized, in vitro, with bacteriophage **T7 RNA polymerase**. The **analogue** -containing precursor tRNAs were authentic substrates for the two splicing enzymes that were tested (endonuclease and ligase), and they formed specific covalent bonds with ligase when they were irradiated with long-wavelength ultraviolet light. We have determined the position of three major cross-links and one minor cross-link on precursor tRNAPhe that were located within the intron and near the 3' splice site. On the basis of these data, we present a model for the in vivo splicing reaction of yeast precursor tRNAs.

1988

the release of **RNA**. Mismatches in the templates at -6 to +1 allowed for efficient termination, unlike those upstream of the sequence. The upstream module (from -15 to -9 approximately -7) functions as a duplex. Pausing of the SP6 elongation complex at the termination site was detected when **RNA** release was suppressed by the incorporation of 5-bromo-UMP, and it was dependent on the upstream module. Results of single-round SP6 transcriptions using 3'-deoxynucleotides and immobilized templates indicated that **RNA** was not released from the elongation complexes halted at the termination site on the template variants carrying mutations in the upstream or downstream module, whereas such complexes on the wild type template were dissociated. Thus, halting or simple pausing was not sufficient for termination even when the downstream module was intact. The upstream module appears to mediate such conformation change necessary for termination.

11/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09979611 98409449 PMID: 9737873
Identifying **RNA** minor groove tertiary contacts by nucleotide **analogue** interference mapping with N2-methylguanosine.
Ortoleva-Donnelly L; Kronman M; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
Biochemistry (UNITED STATES) Sep 15 1998, 37 (37) p12933-42, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Nucleotide **analogue** interference mapping (NAIM) is a general biochemical method that rapidly identifies the chemical groups important for **RNA** function. In principle, NAIM can be extended to any nucleotide that can be incorporated into an in vitro transcript by an **RNA** polymerase. Here we report the synthesis of 5'-O-(1-thio)-N2-methylguanosine triphosphate (m2GalphaS) and its incorporation into two reverse splicing forms of the Tetrahymena group I intron using a mutant form of **T7 RNA** polymerase. This **analogue** replaces one proton of the N2 exocyclic amine with a methyl group, but is as stable as guanosine (G) for **secondary structure** formation. We have identified three sites of m2GalphaS interference within the Tetrahymena intron: G22, G212, and G303. All three of these guanosine residues are known to utilize their exocyclic amino groups to participate in tertiary hydrogen bonds within the ribozyme **structure** . Unlike the interference pattern with the phosphorothioate of inosine (IalphaS, an **analogue** that deletes the N2 amine of G), m2GalphaS substitution did not cause interference at positions attributable to **secondary** structural stability effects. Given that the **RNA** minor groove is likely to be widely used for helix packing, m2GalphaS provides an especially valuable reagent to identify **RNA** minor groove tertiary contacts in less well-characterized RNAs.

11/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09639618 98060914 PMID: 9396824
Single substitutions of phosphorothioates in the HDV ribozyme G73 define regions necessary for optimal self-cleaving activity.
Prabhu N S; Dinter-Gottlieb G; Gottlieb P A
Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104, USA.
Nucleic acids research (ENGLAND) Dec 15 1997, 25 (24) p5119-24,

ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: AI31821; AI; NIAID; GM52033; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Phosphorothioate (NTPalphaS) **analogues** were incorporated into the HDV genomic ribozyme by transcription with **T7** polymerase. The introduction of a sulfur in place of the pro-Rp oxygen at the phosphate 5'to positions A64, A63, A43, U27, G62, C61, C44, C41, C22and C21appeared to inhibit self-cleavage activity of the G73 genomic ribozyme. Except for position C22, elevated levels of Mg2+rescued the reaction to various extents. When the sites were identified in the **RNA** sequence, they were clustered in three distinct regions that, in the **secondary structure** models, are predicted to be primarily single-stranded. Two of these regions have been proposed to form extensive interactions that are thought to involve a homopurine base pair. The third region is thought to be directly associated with assembly of the cleavage site.

11/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09068615 96419174 PMID: 8821947
Purification of the Tn10-specified tetracycline efflux antiporter TetA in a native state as a polyhistidine fusion protein.
Aldema M L; McMurry L M; Walmsley A R; Levy S B
Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.
Molecular microbiology (ENGLAND) Jan 1996, 19 (1) p187-95, ISSN 0950-382X Journal Code: 8712028
Contract/Grant No.: AI30646; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The bacterial tetracycline-resistance determinant from Tn10 encodes a 43 kDa membrane protein, TetA, responsible for active efflux of tetracyclines. The tetA gene was cloned behind a **T7** promoter/lac operator in a plasmid that provided fusion of TetA to a polyhistidine-carboxy terminal tail. A second plasmid provided a regulated **T7 RNA** polymerase. The specific activity of the TetA fusion protein was between 10-40% that of the wild-type protein as assayed by tetracycline resistance in cells and by transport in membrane vesicles. The fusion protein, overproduced approximately 3-13-fold, was purified by nickel chelation chromatography. Calculations from circular dichroism spectra of the purified protein solubilized in dodecylmaltoside gave an alpha-helix content of 54-64%, close to the 68% predicted from the amino acid sequence by hydropathy analysis (12 membrane-spanning helices) for the native protein in the membrane bilayer. Fluorescence studies showed binding activity of the purified protein to its substrate, the tetracycline **analogue** 13-(cyclopentylthio)-5-hydroxy-6-alpha-deoxytetracycline. These findings suggested that the purified protein was in a native state.

11/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07221661 92144548 PMID: 1737000
Binding of protein synthesis initiation factor 4E to oligoribonucleotides: effects of cap accessibility and **secondary structure**.
Carberry S E; Friedland D E; Rhoads R E; Goss D J
Department of Chemistry, Hunter College of the City University of New

York 10021-5024.
Biochemistry (UNITED STATES) Feb 11 1992, 31 (5) p1427-32, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM 20818; GM; NIGMS; RR-0307; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The binding of rabbit globin mRNA to the 25-kDa cap binding protein eIF-4E from human erythrocytes was found to be 5.3-fold stronger than the binding of the cap **analogue** m7GpppG to eIF-4E [Gross et al. (1990) Biochemistry 29, 5008-5012]. In order to investigate whether this effect is due to the longer sequence of nucleotides in globin mRNA or to other features such as cap accessibility or **secondary structure**, oligoribonucleotide **analogues** of rabbit alpha-globin mRNA were synthesized by **T7 RNA** polymerase from a synthetic oligodeoxynucleotide template in the presence of m7GpppG; these oligoribonucleotide **analogues** possess varying degrees of cap accessibility and **secondary structure**. Equilibrium association constants for the interaction of these oligoribonucleotides and purified human erythrocyte eIF-4E were obtained from direct fluorescence titration experiments. The data indicate that while the presence of the m7G cap is required for efficient recognition by eIF-4E, the cap need not be completely sterically accessible, since other structural features within the mRNA also influence binding.

11/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07076218 92011516 PMID: 1655731
Spin-labeled nucleotide substrates for DNA-dependent **RNA** polymerase from Escherichia coli.
Tyagi S C
Department of Biochemistry and Cell Biology, State University of New York, Stony Brook 11794-5215.
Journal of biological chemistry (UNITED STATES) Sep 25 1991, 266 (27) p17936-40, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
New spin-labeled **analogs** of nucleoside triphosphates, 8-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)adenosine 5'-triphosphate ((8-AmTEMPO)ATP) and 5-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)uridine 5'-triphosphate ((5-AmTEMPO)UTP), with the probe 4-amino(2,2,6,6-tetramethylpiperidine-N-oxyl) (4-AmTEMPO) attached to C-8 of ATP and C-5 of UTP via a **secondary** amine bond, were synthesized in 50 and 40% yield, respectively. These **analogs** showed a single spot by thin layer chromatographic analysis. The absorption spectra of (8-Am-TEMPO)ATP and (5-AmTEMPO)UTP exhibit maxima at 310 and 265 nm, respectively; their X-band EPR spectra have a typical three-line pattern with lines at 3,221, 3,239, and 3,257 Gauss. The intensity ratios for mid to high field lines of the EPR derivative lines were found to be 1.03 +/- 0.02, 1.08 +/- 0.04, and 1.15 +/- 0.07 for 4-AmTEMPO, (8-AmTEMPO)ATP, and (5-AmTEMPO)UTP, respectively. The immobilization of 4-AmTEMPO bound to C-8 of ATP or bound to C-5 of UTP was observed to be 5 and 11%, respectively, as compared with free 4-AmTEMPO. The initial velocity (s-1) of [3H]UMP incorporation into **RNA** in the presence of [3H]UTP, CTP, GTP, and (8-AmTEMPO)ATP or ATP was measured. The percent incorporation of (8-AmTEMPO)ATP into **RNA** product by Escherichia coli **RNA** polymerase using various DNA templates is 68, 66, and 61% for pAR1435 (plasmid containing A1 promoter from **T7** DNA), calf thymus DNA, and poly(dA-dT) respectively, as compared with ATP incorporation. The polymerase-catalyzed reaction of

(8-AmTEMPO)ATP with (3'-OCH3)UTP yielded 5'-triphosphate delta-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)adenylyl (3'-5')3'-methoxy uridine in the presence of poly(dA-dT). The **structure** of this spin-labeled dinucleotide was identified by paper chromatographic analysis of the products of phosphodiesterase digestion. These **analogs** also can be used for the study by EPR spectroscopy of the dynamics of gene transcription catalyzed by **RNA** polymerases or of other nucleotide-utilizing enzymes.

exposure to many different stress conditions results in rapid and strong sigma(S) induction. Consequently, transcription of numerous sigma(S)-dependent genes is activated, many of which encode gene products with stress-protective functions. Multiple signal integration in the control of the cellular sigma(S) level is achieved by rpoS transcriptional and translational control as well as by regulated sigma(S) proteolysis, with various stress conditions differentially affecting these levels of sigma(S) control. Thus, a **reduced** growth rate results in increased rpoS transcription whereas high osmolarity, low temperature, acidic pH, and some late-log-phase signals stimulate the translation of already present rpoS mRNA. In addition, carbon starvation, high osmolarity, acidic pH, and high temperature result in stabilization of sigma(S), which, under nonstress conditions, is degraded with a half-life of one to several minutes. Important cis-regulatory determinants as well as trans-acting regulatory factors involved at all levels of sigma(S) regulation have been identified. rpoS translation is controlled by several proteins (Hfq and HU) and small regulatory RNAs that probably affect the **secondary structure** of rpoS mRNA. For sigma(S) proteolysis, the response regulator RssB is essential. RssB is a specific direct sigma(S) recognition factor, whose affinity for sigma(S) is modulated by phosphorylation of its receiver domain. RssB delivers sigma(S) to the ClpXP protease, where sigma(S) is unfolded and completely degraded. This review summarizes our current knowledge about the molecular functions and interactions of these components and tries to establish a framework for further research on the mode of multiple signal input into this complex regulatory system.

4/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13522989 22195892 PMID: 12206755
Polyadenylation can regulate ColE1 type plasmid copy number independently of any effect on RNAI decay by decreasing the interaction of antisense RNAI with its RNAII target.
Xu Feng; Gaggero Carina; Cohen Stanley
Department of Genetics, Stanford University, CA 94305-5120, Stanford, USA
Plasmid (United States) Jul 2002, 48 (1) p49, ISSN 0147-619X
Journal Code: 7802221
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Replication of ColE1-type plasmids is regulated by RNAI, an antisense **RNA** that interacts with the replication pre-primer, RNAII. Exonucleolytic attack at the 3(') end of RNAI is impeded in pcnB mutant bacteria, which lack poly(A) **polymerase** I-the principal **RNA** polyadenylase of E. coli; this leads to accumulation of an RNAI decay intermediate (RNAI(-5)) and dramatic **reduction** of the plasmid copy number. Here, we report that polyadenylation can also affect RNAI-mediated control of plasmid DNA replication by inhibiting interaction of RNAI(-5) with RNAII. We show that mutation of the host pcnB gene profoundly affects the plasmid copy number, even under experimental conditions that limit the effects of polyadenylation on RNAI(-5) decay. Moreover, poly(A) tails interfere with RNAI/RNAII interaction in vitro without producing any detectable alteration of RNAI **secondary structure**. Our results establish the existence of a previously undetected mechanism by which **RNA** polyadenylation can control plasmid copy number.

4/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13494740 22129254 PMID: 12134040
Mutations of the RNase H C helix of the Moloney murine leukemia virus

reverse transcriptase reveal defects in polypurine tract recognition.
Lim David; Orlova Marianna; Goff Stephen P
Integrated Program in Cellular, Molecular and Biophysical Studies, Howard Hughes Medical Institute, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA.
Journal of virology (United States) Aug 2002, 76 (16) p8360-73, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA 30488; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Both the RNase H domain of Moloney murine leukemia virus (Mo-MLV) reverse transcriptase (RT) and Escherichia coli RNase H possess a positively charged alpha-helix (C helix) and a loop that are not present in the RNase H domains of human immunodeficiency virus (HIV) RT or avian sarcoma virus RT. Although a mutant Mo-MLV RT lacking the C helix (DeltaC RT) retains DNA **polymerase** activity on homopolymeric substrates and partial RNase H activity, reverse transcription of the viral **RNA** genome in vivo is defective. To identify the essential features of the C helix, a panel of Mo-MLV RT mutants was generated. Analyses of these mutant viruses revealed the importance of residues H594, I597, R601, and G602. The mutants were tested for their ability to synthesize viral DNA after acute infections and to form proper 5' and 3' viral DNA ends. The mutant RTs were tested in vitro for exogenous RT activity, minus-strand strong-stop DNA synthesis in endogenous RT reactions, nonspecific RNase H activity, and finally, proper cleavage at the polypurine tract-U3 junction. The R601A mutant was the most defective mutant both in vivo and in vitro and possessed very little RNase H activity. The H594A, I597A, and G602A mutants had significant **reductions** in RNase H activity and in their rates of viral replication. Many of the mutants formed improper viral DNA ends and were less efficient in PPT-U3 recognition and cleavage in vitro. The data show that the C helix plays a crucial role for overall RNase H cleavage activity. The data also suggest that the C helix may play an important role in polypurine tract recognition and proper formation of the plus-strand DNA's 5' end.

4/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13420655 22080417 PMID: 12083828
Mutations in NS5B **polymerase** of hepatitis C virus: impacts on in vitro enzymatic activity and viral **RNA** replication in the subgenomic replicon cell culture.
Cheney I Wayne; Naim Suhaila; Lai Vicky C H; Dempsey Shannon; Bellows Daniel; Walker Michelle P; Shim Jae Hoon; Horscroft Nigel; Hong Zhi; Zhong Weidong
Drug Discovery, Ribapharm, Inc., 3300 Hyland Avenue, Costa Mesa, California 92626, USA.
Virology (United States) Jun 5 2002, 297 (2) p298-306, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hepatitis C virus (HCV) nonstructural protein 5B (NS5B) is an **RNA** -dependent **RNA polymerase** (RdRp) essential for virus replication. Several consensus sequence motifs have been identified in NS5B, some of which have been shown to be critical for its enzymatic activity. A unique beta-hairpin **structure** located between amino acids 443 and 454 in the thumb subdomain has also been shown to play an important role in ensuring terminal initiation of **RNA** synthesis in vitro. However, the importance of these sequence and structural elements in viral

RNA replication in infected cells has not been established, mainly due to the lack of a reliable cell culture system for HCV. In this study, we investigated the effect of several single amino acid substitutions and beta-hairpin truncations in NS5B on viral **RNA** replication by using the subgenomic replicon cell culture system. A strong correlation between in vitro **polymerase** activity and viral **RNA** replication was observed with most of the substitutions. Interestingly, truncations of the beta-hairpin (by four and eight amino acid residues, respectively), which did not **reduce** the in vitro enzymatic activity, completely abolished the ability of the replicon **RNA** to replicate in Huh-7 cells, demonstrating its essential role in viral **RNA** replication. Furthermore, a conservative substitution in motif D, from an arginine residue (AMTR(345)), which is conserved among all HCV isolates, to a lysine residue, resulted in significant improvements in both transient **RNA** replication and colony formation efficiencies. This result also correlates with a previous observation that the enzymatic activity of NS5B increased by about 50% when the same NS5B substitution was introduced (V. Lohmann, F. Korner, U. Herian, and R. Bartenschlager, J. Virol. 1997, 71, 8416-8428).

4/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13402838 22135302 PMID: 12139478
Effect of Tetrahydrocortisol-Apolipoprotein A-I Complex on the **Secondary Structure** of Eukaryotic DNA and Its Interaction with **RNA-Polymerase**.
Panin L E; Gimautdinova O I; Kuznetsov P A; Akimzhanova M V; Tuzikov F V
Research Institute of Biochemistry, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, 630117, Russia. ibch@cyber.ma.nsc.ru
Biochemistry. Biokhimii a (United States) Jul 2002, 67 (7) p790-4, ISSN 0006-2979 Journal Code: 0376536
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
The in vitro effect of tetrahydrocortisol-apolipoprotein A-I complex on native adult rat liver DNA results in the formation of S1 nuclease sensitive fragments that are irregularly distributed throughout a genome. Low-angle X-ray scattering showed that after the interaction with the tetrahydrocortisol-apolipoprotein A-I complex, DNA can bind to **RNA-polymerase** with a high and dose-dependent cooperativity. This indicates that the effect of tetrahydrocortisol-apolipoprotein A-I complex on **secondary** eukaryotic DNA **structure** causes a local denaturation of the double helix, promoting high cooperativity of binding to **RNA-polymerase**. The **reduced** form of the hormone, tetrahydrocortisol, previously considered as an inactive metabolite, when complexed with apolipoprotein A-I, promotes a biological function similar to that of a transcription factor.

4/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13375542 22077261 PMID: 12081963
Group I self-splicing intron in the recA gene of Bacillus anthracis.
Ko Minsu; Choi Hyang; Park Chankyu
National Creative Research Initiative Center for Behavioral Genetics, Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Yusong-Ku, Taejon 305-701, Republic of Korea.
Journal of bacteriology (United States) Jul 2002, 184 (14) p3917-22, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Self-splicing introns are rarely found in bacteria and bacteriophages. They are classified into group I and II according to their structural features and splicing mechanisms. While the group I introns are occasionally found in protein-coding regions of phage genomes and in several tRNA genes of cyanobacteria and proteobacteria, they had not been found in protein-coding regions of bacterial genomes. Here we report a group I intron in the recA gene of Bacillus anthracis which was initially found by DNA sequencing as an intervening sequence (IVS). By using reverse transcriptase PCR, the IVS was shown to be removable from the recA precursor mRNA for RecA that was being translated in E. coli. The splicing was visualized in vitro with labeled free GTP, indicating that it is a group I intron, which is also implied by its predicted **secondary structure** . The RecA protein of B. anthracis expressed in E. coli was functional in its ability to complement a recA defect. When recA-negative E. coli cells were irradiated with UV, the Bacillus RecA **reduced** the UV susceptibility of the recA mutant, regardless of the presence of intron.

4/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13258806 21988165 PMID: 11877386
Structural determinants of BRCA1 translational regulation.
Sobczak Krzysztof; Krzyzosiak Wlodzimierz J
Laboratory of Cancer Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland.
Journal of biological chemistry (United States) May 10 2002, 277 (19) p17349-58, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The BRCA1 gene is involved in sporadic breast and ovarian cancer mainly through **reduced** expression. BRCA1 mRNAs containing different leader sequences show different patterns of expression. In a normal mammary gland mRNA with a shorter leader sequence, 5'-UTRa is expressed only, whereas in breast cancer tissue mRNA with a longer leader, 5'-UTRb is expressed also. We show that the translation efficiency of transcripts containing 5'-UTRb is 10 times lower than those containing 5'-UTRa. The structures of 5'-UTRa and 5'-UTRb were determined by chemical and enzymatic probing aided by a new method developed for monitoring the number of co-existing stable conformers. Specific factors responsible for **reduced** translation of mRNA containing 5'-UTRb were determined using a variety of transcripts with mutations in the leader sequence. These factors include a stable **secondary structure** formed by truncated Alu element and upstream AUG codons. The novel mechanism by which BRCA1 may be involved in sporadic breast and ovarian cancer is proposed. It is based on the expression patterns of BRCA1 mRNAs and differences in their translatability. According to this mechanism the deregulation of the BRCA1 transcription in cancer, resulting in a higher proportion of translationally inhibited transcripts containing 5'-UTRb, contributes to the decrease in the BRCA1 protein observed in sporadic breast and ovarian cancers.

4/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13054924 21908220 PMID: 11911360
Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1.

Frolova Ludmila; Seit-Nebi Alim; Kisselev Lev
Engelhardt Institute of Molecular Biology, Moscow, Russia.
RNA (New York, N.Y.) (United States) Feb 2002, 8 (2) p129-36, ISSN 1355-8382 Journal Code: 9509184
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Class-1 polypeptide chain release factors (RFs) play a key role in translation termination. Eukaryotic (eRF1) and archaeal class-1 RFs possess a highly conserved Asn-Ile-Lys-Ser (NIKS) tetrapeptide located at the N-terminal domain of human eRF1. In the three-dimensional **structure**, NIKS forms a loop between helices. The universal occurrence and exposed nature of this motif provoke the appearance of hypotheses postulating an essential role of this tetrapeptide in stop codon recognition and ribosome binding. To approach this problem experimentally, site-directed mutagenesis of the NIKS (positions 61-64) in human eRF1 and adjacent amino acids has been applied followed by determination of release activity and ribosome-binding capacity of mutants. Substitutions of Asn61 and Ile62 residues of the NIKS cause a decrease in the ability of eRF1 mutants to promote termination reaction in vitro, but to a different extent depending on the stop codon specificity, position, and nature of the substituting residues. This observation points to a possibility that Asn-Ile dipeptide modulates the specific recognition of the stop codons by eRF1. Some replacements at positions 60, 63, and 64 cause a negligible (if any) effect in contrast to what has been deduced from some current hypotheses predicting the **structure** of the termination codon recognition site in eRF1. **Reduction** in ribosome binding revealed for Ile62, Ser64, Arg65, and Arg68 mutants argues in favor of the essential role played by the right part of the NIKS loop in interaction with the ribosome, most probably with ribosomal **RNA**.

4/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12769906 21601725 PMID: 11602578
HIV-1 nucleocapsid protein and the **secondary structure** of the binary complex formed between tRNA(Lys.3) and viral **RNA** template play different roles during initiation of (-) strand DNA reverse transcription.
Rong L; Liang C; Hsu M; Guo X; Roques B P; Wainberg M A
McGill AIDS Centre, Lady Davis Institute-Jewish General Hospital, Montreal, Quebec H3T 1E2, Canada.
Journal of biological chemistry (United States) Dec 14 2001, 276 (50) p47725-32, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In human immunodeficiency virus type 1 (HIV-1), the tRNA(Lys.3) primer and viral **RNA** template can form a specific complex that is characterized by extensive inter- and intramolecular interactions. Initiation of reverse transcription from this complex has been shown to be distinguished from subsequent elongation by early pausing events, such as at the +1 and +3 nucleotide positions. One major concern regarding the biological relevance of these results is that most kinetic studies of HIV-1 reverse transcription have been performed using tRNA(Lys.3)-viral (v) **RNA** complexes that were formed by heat annealing. In contrast, tRNA(Lys.3) in viruses is placed onto the primer binding site by nucleocapsid (NC) sequences of the Gag protein. In this study, we have further characterized the initiation features of reverse transcription in the presence of HIV-1 NC protein. In contrast to results obtained with a heat-annealed tRNA(Lys.3).vRNA complex, we found that polymerization reactions catalyzed by HIV-1 reverse transcriptase did not commonly pause

at the +1 nucleotide position when a NC-annealed **RNA** complex was used, and that this was true regardless whether NC was actually still present during reverse transcription. This activity of NC required both zinc finger motifs, as demonstrated by experiments that employed zinc finger-mutated forms of NC protein (H23C NC and ddNC), supporting the involvement of the zinc fingers in the **RNA** chaperone activity of NC. However, NC was not able to help reverse transcriptase to escape the +3 pausing event. Mutagenesis of a stem **structure** within the tRNA(Lys.3). vRNA complex led to disappearance of the +3 pausing event as well as to significantly **reduced** rates of reverse transcription. Thus, this stem **structure** is essential for optimal reverse transcription, despite its role in promotion of the +3 pausing event.

4/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11358707 21437504 PMID: 11553235
16S-23S rDNA intergenic spacer and 23S rDNA of anaerobic ammonium-oxidizing bacteria: implications for phylogeny and in situ detection.
Schmid M; Schmitz-Esser S; Jetten M; Wagner M
Lehrstuhl fur Mikrobiologie, Technische Universitat Munchen, Am Hochanger 4, D-85350 Freising, Germany.
Environmental microbiology (England) Jul 2001, 3 (7) p450-9, ISSN 1462-2912 Journal Code: 100883692
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Recently, anaerobic ammonium-oxidizing bacteria (AAOB) were identified by comparative 16S rDNA sequence analysis as a novel, deep-branching lineage within the Planctomycetales. This lineage consists currently of only two, not yet culturable bacteria which have been provisionally described as Candidatus 'Brocadia anammoxidans' and Candidatus 'Kuenenia stuttgartiensis'. In this study, a large fragment of the rDNA operon, including the 16S rDNA, the intergenic spacer region (ISR) and approximately 2 000 bases of the 23S rDNA, was **polymerase** chain reaction (PCR) amplified, cloned and sequenced from both AAOB. The retrieved 16S rDNA sequences of both species contain an insertion at helix 9 with a previously overlooked pronounced **secondary structure** (new subhelices 9a and 9b). This insertion, which is absent in all other known prokaryotes, is detectable by fluorescence in situ hybridization (FISH) and thus present in the mature 16S rRNA. In contrast with the genera Pirellula, Planctomyces and Gemmata that possess unlinked 16S and 23S rRNA genes, both AAOB have the respective genes linked together by an ISR of approximately 450 bp in length. Phylogenetic analysis of the obtained 23S rRNA-genes confirmed the deep branching of the AAOB within the Planctomycetales and allowed the design of additional specific FISH probes. Remarkably, the ISR of the AAOB also could be successfully detected by FISH via simultaneous application of four monolabelled oligonucleotide probes. Quantitative FISH experiments with cells of Candidatus 'Brocadia anammoxidans' that were inhibited by exposure to oxygen for different time periods demonstrated that the concentration of transcribed ISR reflected the activity of the cells more accurately than the 16S or 23S rRNA concentration. Thus the developed ISR probes might become useful tools for in situ monitoring of the activity of AAOB in their natural environment.

4/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11285066 21337024 PMID: 11443520
RNA editing of the 5-HT(2C) receptor is **reduced** in

schizophrenia.
Sodhi M S; Burnet P W; Makoff A J; Kerwin R W; Harrison P J
University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK. monsheel.sodhi@psych.ox.ac.uk
Molecular psychiatry (England) Jul 2001, 6 (4) p373-9, ISSN 1359-4184 Journal Code: 9607835
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
5-HT(2C) receptor (5HT(2C)R, serotonin-2C) **RNA** undergoes editing to produce several receptor variants, some with pharmacological differences. This investigation comprised two parts: the characterisation of 5-HT(2C)R **RNA** editing in a larger human control sample than previously examined, and a comparative study in subjects with schizophrenia. **Secondary structure** analysis of the putative edited region of the human 5-HT(2C)R gene predicted the existence of a double stranded (ds) **RNA** loop, essential for **RNA** editing in this receptor. **RNA** was then extracted from frontal cortex of five controls and five subjects with schizophrenia. RT-PCR products of the edited region were cloned and sequenced (n = 100). **Reduced RNA** editing, increased expression of the unedited 5-HT(2C-INI) isoform in schizophrenia (P = 0.001) and decreased expression of the 5-HT(2C-VSV) and 5-HT(2C-VNV) isoforms were detected in the schizophrenia group. In addition, two novel mRNA edited variants were identified: 5-HT(2C-MNI) and 5-HT(2C-VDI). Screening of the 5-HT(2C)R gene did not reveal any mutations likely to disrupt the dsRNA loop, suggesting that the **reduced RNA** editing in schizophrenia may instead be caused by altered activity of the editing enzyme(s). Since the unedited 5-HT(2C-INI) is more efficiently coupled to G proteins than the other isoforms, its increased expression in schizophrenia may lead to enhanced 5-HT(2C)R-mediated effects. The results also illustrate that potentially important receptor alterations may occur in schizophrenia which are not detectable merely in terms of receptor abundance.

4/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11248085 21278779 PMID: 11384232
Identification of a putative binding site for [2',5'-bis-O-(tert-butyldimethylsilyl)-beta-D-ribofuranosyl]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)thymine (TSAO) derivatives at the p51-p55 interface of HIV-1 reverse transcriptase.
Rodriguez-Barrios F; Perez C; Lobaton E; Velazquez S; Chamorro C; San-Felix A; Perez-Perez M J; Camarasa M J; Pelemans H; Balzarini J; Gago F
Departamento de Farmacologia, Universidad de Alcala, E-28871 Alcala de Henares, Madrid, Spain.
Journal of medicinal chemistry (United States) Jun 7 2001, 44 (12) p1853-65, ISSN 0022-2623 Journal Code: 9716531
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A binding site for TSAO-m(3)T at the interface between the p66 and p51 subunits of HIV-1 reverse transcriptase (RT) and distinct from that of "classical" HIV-1 non-nucleoside inhibitors is proposed. The feasibility of the binding mode was assessed by carrying out nanosecond molecular dynamics simulations for the complexes of TSAO-m(3)T with **reduced** models of both the wild-type enzyme and a more sensitive R172A mutant. The molecular model is in agreement with a previous proposal, with known **structure** -activity and mutagenesis data for this unique class of inhibitors, and also with recent biochemical evidence indicating that TSAO analogues can affect enzyme dimerization. The relative importance of residues involved in dimer formation and TSAO-RT complex stabilization was assessed by a

combination of surface area accessibility, molecular mechanics, and continuum electrostatics calculations. A **structure**-based modification introduced into the lead compound yielded a new derivative with improved antiviral activity.

4/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11248063 21278758 PMID: 11385363
Reverse transcriptase template switching during reverse transcriptase-**polymerase** chain reaction: artificial generation of deletions in ribonucleotide **reductase** mRNA.
Mader R M; Schmidt W M; Sedivy R; Rizovski B; Braun J; Kalipciyan M; Exner M; Steger G G; Mueller M W
Department of Internal Medicine I, Division of Oncology, University of Vienna, Austria.
Journal of laboratory and clinical medicine (United States) Jun 2001, 137 (6) p422-8, ISSN 0022-2143 Journal Code: 0375375
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Using reverse transcriptase-**polymerase** chain reaction (RT-PCR), we have recently described a bona fide deletion within the coding sequence of the large subunit of ribonucleotide **reductase** (R1) mRNA in colon cancer. Consecutive studies have raised questions about the nature of this phenomenon, because the corresponding genomic alteration at the DNA level or an aberrant protein could not be detected. Thus we considered an in vitro artifact during RT-PCR as a possible explanation for this observation. In contrast to reverse transcriptase, Taq DNA **polymerase** or C. therm DNA **polymerase** did not generate the aberrant product, suggesting the demand for the template switching activity intrinsic to retroviral reverse transcriptases. In fact, virtually the same deletion was observed in RT-PCR experiments when in vitro transcribed R1 mRNA was used. Considering structural prerequisites for template switching within R1 mRNA, we show that two direct repeats adjacent to a strong stem-loop **secondary structure** flank the deleted region of 1851 base pairs. Because several mRNAs encoding proteins of clinical and diagnostic importance fulfill these criteria, template switching enhances the potential risk of observing artifacts when interpreting results from RT-PCR studies. As shown in the present example, this may involve the artificial generation and the misinterpretation of PCR fragments amplified from targets relevant to tumor biology or cancer pharmacology. As a possible solution, one-step PCR with C. therm **polymerase** should be considered. This **polymerase** eliminates the artificial generation of aberrant mRNA signals observed during cDNA synthesis.

4/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11154328 21172431 PMID: 11274475
Thermal and urea-induced unfolding in T7 **RNA polymerase**: calorimetry, circular dichroism and fluorescence study.
Griko Y; Sreerama N; Osumi-Davis P; Woody R W; Woody A Y
Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218-2685, USA.
Protein science : a publication of the Protein Society (United States) Apr 2001, 10 (4) p845-53, ISSN 0961-8368 Journal Code: 9211750
Contract/Grant No.: GM23697; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Structural changes in T7 **RNA polymerase** (T7RNAP) induced by temperature and urea have been studied over a wide range of conditions to obtain information about the structural organization and the stability of the enzyme. T7RNAP is a large monomeric enzyme (99 kD). Calorimetric studies of the thermal transitions in T7RNAP show that the enzyme consists of three cooperative units that may be regarded as structural domains. Interactions between these structural domains and their stability strongly depend on solvent conditions. The unfolding of T7RNAP under different solvent conditions induces a highly stable intermediate state that lacks specific tertiary interactions, contains a significant amount of residual **secondary structure** , and undergoes further cooperative unfolding at high urea concentrations. Circular dichroism (CD) studies show that thermal unfolding leads to an intermediate state that has increased beta-sheet and **reduced** alpha-helix content relative to the native state. Urea-induced unfolding at 25 degrees C reveals a two-step process. The first transition centered near 3 M urea leads to a plateau from 3.5 to 5.0 M urea, followed by a second transition centered near 6.5 M urea. The CD spectrum of the enzyme in the plateau region, which is similar to that of the enzyme thermally unfolded in the absence of urea, shows little temperature dependence from 15 degrees to 60 degrees C. The second transition leads to a mixture of poly(Pro)II and unordered conformations. As the temperature increases, the ellipticity at 222 nm becomes more negative because of conversion of poly(Pro)II to the unordered conformation. Near-ultraviolet CD spectra at 25 degrees C at varying concentrations of urea are consistent with this picture. Both thermal and urea denaturation are irreversible, presumably because of processes that follow unfolding.

4/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11143937 21150462 PMID: 11251833
What makes an Escherichia coli promoter sigma(S) dependent? Role of the -13/-14 nucleotide promoter positions and region 2.5 of sigma(S).
Becker G; Hengge-Aronis R
Institut fur Biologie - Mikrobiologie, Freie Universitat Berlin, Konigin-Luise-Str. 12-16, 14195 Berlin, Germany.
Molecular microbiology (England) Mar 2001, 39 (5) p1153-65, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The sigmaS and sigma70 subunits of Escherichia coli **RNA polymerase** recognize very similar promoter sequences. Therefore, many promoters can be activated by both holoenzymes in vitro. The same promoters, however, often exhibit distinct sigma factor selectivity in vivo. It has been shown that high salt conditions, **reduced** negative supercoiling and the formation of complex nucleoprotein structures in a promoter region can contribute to or even generate sigmaS selectivity. Here, we characterize the first positively acting sigmaS-selective feature in the promoter sequence itself. Using the sigmaS-dependent csiD promoter as a model system, we demonstrate that C and T at the -13 and -14 positions, respectively, result in strongest expression. We provide allele-specific suppression data indicating that these nucleotides are contacted by K173 in region 2.5 of sigmaS. In contrast, sigma70, which features a glutamate at the corresponding position (E458), as well as the sigmaS(K173E) variant, exhibit a preference for a G(-13). C(-13) is highly conserved in sigmaS-dependent promoters, and additional data with the osmY promoter demonstrate that the K173/C(-13) interaction is of general importance. In conclusion, our data demonstrate an important role for region 2.5 in sigmaS in transcription initiation. Moreover, we propose a

consensus sequence for a sigmaS-selective promoter and discuss its emergence and functional properties from an evolutionary point of view.

4/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11105980 21132706 PMID: 11243267
Changes in the community **structure** of ammonia-oxidizing bacteria during **secondary** succession of calcareous grasslands.
Kowalchuk G A; Stienstra A W; Heilig G H; Stephen J R; Woldendorp J W
Netherlands Institute for Ecology, Centre for Terrestrial Ecology, Heteren. gkowal@cto.nioo.knaw.nl
Environmental microbiology (England) Feb 2000, 2 (1) p99-110, ISSN 1462-2912 Journal Code: 100883692
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The community **structure** of beta-subclass Proteobacteria ammonia-oxidizing bacteria was determined in semi-natural chalk grassland soils at different stages of **secondary** succession. Both culture-mediated (most probable number; MPN) and direct nucleic acid-based approaches targeting genes encoding 16S rRNA and the AmoA subunit of ammonia monooxygenase were used. Similar shifts were detected in the composition of the ammonia oxidizer communities by both culture-dependent and independent approaches. A predominance of Nitrosospira sequence cluster 3 in early successional fields was replaced by Nitrosospira sequence cluster 4 in late successional fields. The rate of this shift differed between the two areas examined. This shift occurred in a background of relative stability in the dominant bacterial populations in the soil, as determined by domain-level **polymerase** chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Molecular analysis of enrichment cultures obtained using different ammonia concentrations revealed biases towards Nitrosospira sequence cluster 3 or Nitrosospira sequence cluster 4 under high- or low-ammonia conditions respectively. High-ammonia MPNs suggested a decease in ammonia oxidizer numbers with succession, but low-ammonia MPNs and competitive PCR targeting amoA failed to support such a trend. Ammonia turnover rate, not specific changes in plant diversity and species composition, is implicated as the major determinant of ammonia oxidizer community **structure** in successional chalk grassland soils.

4/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10952642 20529920 PMID: 11079536
A novel mitochondrial 12SrRNA point mutation in parkinsonism, deafness, and neuropathy.
Thyagarajan D; Bressman S; Bruno C; Przedborski S; Shanske S; Lynch T; Fahn S; DiMauro S
Center for Parkinson's Disease and Other Movement Disorders, ·New York, NY, USA.
Annals of neurology (UNITED STATES) Nov 2000, 48 (5) p730-6, ISSN 0364-5134 Journal Code: 7707449
Contract/Grant No.: P50-NS38370; NS; NINDS; R01-NS38586; NS; NINDS; R29-NS37345; NS; NINDS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The objective of this study was to determine whether a mitochondrial DNA mutation and defective oxidative phosphorylation are present in a pedigree with maternally inherited sensorineural deafness, levodopa-responsive

parkinsonism, and neuropathy. We sequenced the mitochondrial-encoded ribosomal **RNA**, cytochrome c oxidase, and transfer **RNA** genes by cycle sequencing. A **polymerase** chain reaction-based restriction enzyme assay with mismatched primers was employed to show heteroplasmy of a novel 12SrRNA mutation in the proband and to screen control subjects. Spectrophotometric mitochondrial respiratory chain assays were performed in transformed lymphoblasts from the proband and 12 normal controls. A novel, heteroplasmic, maternally inherited 12SrRNA point mutation (T1095C) was found in the pedigree. Respiratory chain enzyme analysis in cultured lymphocytes from the proband revealed a significant **reduction** in cytochrome c oxidase activity. **Secondary structure** predicts that this mutation disrupts a highly conserved loop in the small subunit ribosomal **RNA**, which is important in the initiation of mitochondrial protein synthesis. The mutation was not found in 270 controls of diverse ethnic origins. We conclude that this mutation is pathogenic and causes an oxidative phosphorylation defect by interfering with mitochondrial protein synthesis.

4/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10866754 20435243 PMID: 10978152
Molecular architecture of the mutagenic active site of human immunodeficiency virus type 1 reverse transcriptase: roles of the beta 8-alpha E loop in fidelity, processivity, and substrate interactions.
Weiss K K; Isaacs S J; Tran N H; Adman E T; Kim B
Department of Microbiology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 672, Rochester, New York 14642, USA.
Biochemistry (UNITED STATES) Sep 5 2000, 39 (35) p10684-94, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: P30-ES07033; ES; NIEHS; R29-GM555000; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is a putative source of the genomic hypermutation that promotes rapid evolution of HIV-1. To understand the molecular strategies that create a highly mutagenic DNA **polymerase** active site in HIV-1 RT, we investigated the roles of four residues in the beta 8-alpha E loop. Gln151, which interacts with the sugar of the incoming dNTP, and Lys154, which interacts with the template, yielded site-directed mutants with increased fidelity, suggesting that these residues are directly involved in the mutagenic architecture of the active site. Mutations at Gln151 and Lys154 also **reduced** processivity. Q151N RT showed enhanced ability to discriminate between TTP and AZT triphosphate, consistent with the observation that the Q151M mutation confers AZT resistance in vivo. Mutations at Gly152 greatly decreased RT activity; molecular modeling suggests that Gly152 is critical for the proper geometric alignment that permits base-pairing of the incoming dNTP with the template. Mutations at Trp153 **reduced** the expression level, and presumably the stability, of RT proteins in bacteria. These observations support the conclusion that interactions of active site residues in the beta 8-alpha E loop with incoming dNTPs and the template are determinants of the accuracy, processivity, and substrate selectivity of HIV-1 RT.

4/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10851995 20385489 PMID: 10929194
Gene conversion of ribosomal DNA in Nicotiana tabacum is associated with

undermethylated, decondensed and probably active gene units.
Lim K Y; Kovarik A; Matyasek R; Bezdek M; Lichtenstein C P; Leitch A R
School of Biological Sciences, Queen Mary and Westfield College, London, UK.
Chromosoma (GERMANY) Jun 2000, 109 (3) p161-72, ISSN 0009-5915 Journal Code: 2985138R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We examined the **structure**, intranuclear distribution and activity of ribosomal DNA (rDNA) in Nicotiana sylvestris (2n = 2x = 24) and N. tomentosiformis (2n = 2x = 24) and compared these with patterns in N. tabacum (tobacco, 2n = 4x = 48). We also examined a long-established N. tabacum culture, TBY-2. Nicotiana tabacum is an allotetraploid thought to be derived from ancestors of N. sylvestris (S-genome donor) and N. tomentosiformis (T-genome donor). Nicotiana sylvestris has three rDNA loci, one locus each on chromosomes 10, 11, and 12. In root-tip meristematic interphase cells, the site on chromosome 12 remains condensed and inactive, while the sites on chromosomes 10 and 11 show activity at the proximal end of the locus only. Nicotiana tomentosiformis has one major locus on chromosome 3 showing activity and a minor, inactive locus on chromosome 11. In N. tabacum cv. 095-55, there are four rDNA loci on T3, S10, S11/t and S12 (S11/t carries a small T-genome translocation). The locus on S12 remains condensed and inactive in root-tip meristematic cells while the others show activity, including decondensation at interphase and **secondary** constrictions at metaphase. Nicotiana tabacum DNA digested with methylcytosine-sensitive enzymes revealed a hybridisation pattern for rDNA that resembled that of N. tomentosiformis and not N. sylvestris. The data indicate that active, undermethylated genes are of the N. tomentosiformis type. Since S-genome chromosomes of N. tabacum show rDNA expression, the result indicates rDNA gene conversion of the active rDNA units on these chromosomes. Gene conversion in N. tabacum is consistent with the results of previous work. However, using primers specific for the S-genome rDNA intergenic sequences (IGS) in the **polymerase** chain reaction (PCR) show that rDNA gene conversion has not gone to completion in N. tabacum. Furthermore, using methylation-insensitive restriction enzymes we demonstrate that about 8% of the rDNA units remain of the N. sylvestris type (from ca. 75% based on the sum of the rDNA copy numbers in the parents). Since the active genes are likely to be of an N. tomentosiformis type, the N. sylvestris type units are presumably contained within inactive loci (i.e. on chromosome S12). Nicotiana sylvestris has approximately three times as much rDNA as the other two species, resulting in much condensed rDNA at interphase. This species also has three classes of IGS, indicating gene conversion has not homogenised repeat length in this species. The results suggest that methylation and/or DNA condensation has **reduced** or prevented gene conversion from occurring at inactive genes at rDNA loci. Alternatively, active undermethylated units may be vulnerable to gene conversion, perhaps because they are decondensed and located in close proximity within the nucleolus at interphase. In TBY-2, restriction enzymes showed hybridisation patterns that were similar to, but different from, those of N. tabacum. In addition, TBY-2 has elevated rDNA copy number and variable numbers of rDNA loci, all indicating rDNA evolution in culture.

4/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10709928 20243668 PMID: 10779603
Structure and sequence variation of the trypanosome spliced leader transcript.
Gibson W; Bingle L; Blendeman W; Brown J; Wood J; Stevens J
School of Biological Sciences, University of Bristol, Bristol, UK. w.gibson@bristol.ac.uk

Molecular and biochemical parasitology (NETHERLANDS) Apr 15 2000, 107 (2) p269-77, ISSN 0166-6851 Journal Code: 8006324
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have assessed the potential of using the spliced leader (SL) or mini-exon gene as a marker for molecular phylogenetic analysis of genus Trypanosoma. A total of 27 trypanosome sequences were compared, 18 of these being newly reported. In contrast to genus Leishmania, we found the non-transcribed spacer region of the SL locus in trypanosomes to be far too variable for informative comparison of all but the most closely related species. At the other extreme, the short (39 nt) SL exon was usually completely conserved and hence uninformative. The SL **RNA** showed variation in both length (97-152 nt) and sequence among different trypanosome species, with most variation occurring in stem-loop II. Consequently, this region could not be aligned with confidence in multiple sequence alignment, severely **reducing** the number of phylogenetically informative nucleotide positions. In computer simulation, most of the SL RNAs readily folded into the 3 stem-loop **secondary structure** predicted previously, but again stem-loop II was highly variable. No obvious correlation could be seen between the length of this stem-loop and trypanosome biology. We conclude that the SL repeat is not an informative phylogenetic marker for long range evolutionary studies of genus Trypanosoma.

4/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10639104 20181861 PMID: 10715203
Constructing high complexity synthetic libraries of long ORFs using in vitro selection.
Cho G; Keefe A D; Liu R; Wilson D S; Szostak J W Szostak J W MA Gen Hosp, Boston
Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114, USA.
Journal of molecular biology (ENGLAND) Mar 24 2000, 297 (2) p309-19, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We present a method that can significantly increase the complexity of protein libraries used for in vitro or in vivo protein selection experiments. Protein libraries are often encoded by chemically synthesized DNA, in which part of the open reading frame is randomized. There are, however, major obstacles associated with the chemical synthesis of long open reading frames, especially those containing random segments. Insertions and deletions that occur during chemical synthesis cause frameshifts, and stop codons in the random region will cause premature termination. These problems can together greatly **reduce** the number of full-length synthetic genes in the library. We describe a strategy in which smaller segments of the synthetic open reading frame are selected in vitro using mRNA display for the absence of frameshifts and stop codons. These smaller segments are then ligated together to form combinatorial libraries of long uninterrupted open reading frames. This process can increase the number of full-length open reading frames in libraries by up to two orders of magnitude, resulting in protein libraries with complexities of greater than 10(13). We have used this methodology to generate three types of displayed protein library: a completely random sequence library, a library of concatemerized oligopeptide cassettes with a propensity for forming amphipathic alpha-helical or beta-strand structures, and a library based on one of the most common enzymatic scaffolds, the alpha/beta (TIM) barrel.

4/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10567872 20097782 PMID: 10634316
The T7 concatemer junction sequence interferes with expression from a downstream T7 promoter in vivo.
Harvey B; Korus M; Goldman E
Graduate School of Biomedical Sciences, University of Medicine & Dentistry of New Jersey, Newark 07103, USA.
Gene expression (UNITED STATES) 1999, 8 (3) p141-9, ISSN 1052-2166 Journal Code: 9200651
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A recently described new signal for transcription termination in vitro by T7 **RNA polymerase** has now been tested in vivo. This signal, identified during transcription of the cloned human preproparathyroid hormone (PTH) gene, is also found in the phage T7 genome, at the concatemer junction (CJ). We introduced the 17-bp concatemer junction sequence at the ends of a test gene and control gene (both derived from T7 gene 9) in a T7 vector previously used to study effects of rare codons on expression. The CJ elements replaced the original vector's RNase III processing sites, and a new T7 promoter was also introduced to drive the downstream (control) gene. We assayed for test and control gene mRNA and protein by direct labeling with [32P]phosphate and [35S]methionine. The altered vector with CJ sequences (pCT1.1) expressed the upstream test gene, but showed poor expression of the downstream control gene. No discrete T7 mRNA bands could be discerned by direct labeling with 32P. A precursor vector with only the control gene in single copy expressed the protein much better, suggesting that the inhibition of control gene expression in pCT1.1 was a result of the upstream CJ element at the 3' end of the test gene. RT-PCR experiments were consistent with readthrough and possibly pausing at CJ. An **RNA** folding program predicts a highly stable **secondary structure** between the upstream CJ element and the control gene's translation start signals. These data support an interpretation that the CJ element is ineffective as a T7 transcription terminator in vivo in this vector, and that **structure** of the readthrough transcript blocks ribosome access to the downstream translation start. The readthrough transcripts are also likely to be less stable than properly terminated or processed T7 mRNA, because levels of test protein expression in pCT1.1 were **reduced** compared to original vector, and basal expression was negligible, while the original codon test vector shows substantial basal expression.

4/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10459145 99454994 PMID: 10523626
A region within the RAP74 subunit of human transcription factor IIF is critical for initiation but dispensable for complex assembly.
Ren D; Lei L; Burton Z F
Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824-1319, USA.
Molecular and cellular biology (UNITED STATES) Nov 1999, 19 (11) p7377-87, ISSN 0270-7306 Journal Code: 8109087
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Human transcription factor IIF (TFIIF) is an alpha(2)beta(2)

heterotetramer of **RNA polymerase** II-associating 74 (RAP74) and RAP30 subunits. Mutagenic analysis shows that the N-terminal region of RAP74 between L155 (leucine at codon 155) and M177 is important for initiation. Mutants in this region have **reduced** activity in transcription, but none are inactive. Single amino acid substitutions at hydrophobic residues L155, W164, I176, and M177 have similar activity to RAP74(1-158), from which all but three amino acids of this region are deleted. Residual activity can be explained because each of these mutants forms a complex with RAP30 and recruits **RNA polymerase** II into the preinitiation complex. Mutants are defective for formation of the first phosphodiester bond from the adenovirus major late promoter but do not appear to have an additional significant defect in promoter escape. Negative DNA supercoiling partially compensates for the defects of TFIIF mutants in initiation, indicating that TFIIF may help to untwist the DNA helix for initiation.

4/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10417469 99396091 PMID: 10468015

Design of potent phosphorothioate antisense oligonucleotides directed to human interleukin 10 gene product and their evaluation of antisense activity in U937 cells.

Arima H; Takahashi M; Aramaki Y; Sakamoto T; Yuda K; Akiyama K; Goto T; Tsuchiya S

School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Japan.

Pharmaceutical research (UNITED STATES) Aug 1999, 16 (8) p1163-71, ISSN 0724-8741 Journal Code: 8406521

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

PURPOSE: The two objectives of this study were to design potent phosphorothioate antisense oligonucleotides (AS-S-oligos) directed against the human interleukin-10 (IL-10) gene product and to reveal the DNA sequence which best activates antisense effects. METHODS: The design of potent AS-S-oligo was performed by using melting temperature (Tm) value of a DNA/**RNA** hybrid calculated by the nearest neighbor method and a **secondary structure** of human IL-10 mRNA suggested by **RNA** folding algorithms. U937 cells were used to estimate the antisense effect of the AS-S-oligos. RESULTS: Of the eight candidates selected as potent AS-S-oligos on the basis of having higher Tm values and favorable **secondary** structures of the IL-10 mRNA, AS-S-oligos directed against the translated (AS367-S-oligo) and 3'-untranslated (AS637-S-oligo) region of IL-10 mRNA showed the strongest inhibitory effects on IL-10 production and this inhibition was dose- and time-dependent. Reverse transcription-**polymerase** chain reaction (RT-PCR) revealed that the antisense effects of AS-S-oligos originated from a specific **reduction** of target IL-10 mRNA by hybridization with AS367- and AS637-S-oligos. In addition, these AS-S-oligos did not affect human tumor necrosis factor-alpha (TNF-alpha) production in the cells stimulated by lipopolysaccharide (LPS). Strong positive correlations between the inhibitory effect of AS-S-oligos on the IL-10 production and not only Tm values calculated by nearest neighbor method but also Tm values determined by absorbance versus temperature profiles were demonstrated except for AS25-S-oligo and AS1249-S-oligo. CONCLUSIONS: These findings suggest AS367- and AS637-S-oligos powerfully inhibit IL-10 production in U937 cells via an antisense mechanism. In addition, it is suggested efficiency of AS-S-oligo directed against the sequence of the target gene product can be explained by these Tm values and the proposed **secondary** structures of the target gene product.

4/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10417368 99403137 PMID: 10471738
Repression of IS200 transposase synthesis by **RNA secondary** structures.
Beuzon C R; Marques S; Casadesus J
Departamento de Genetica, Facultad de Biologia, Universidad de Sevilla, Apartado 1095, E-41080 Sevilla, Spain.
Nucleic acids research (ENGLAND) Sep 15 1999, 27 (18) p3690-5, ISSN 1362-4962 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The IS 200 transposase, a 16 kDa polypeptide encoded by the single open reading frame (ORF) of the insertion element, has been identified using an expression system based on T7 **RNA polymerase**. In wild-type IS 200, two sets of internal inverted repeats that generate **RNA secondary** structures provide two independent mechanisms for repression of transposase synthesis. The inverted repeat located near the left end of IS 200 is a transcriptional terminator that terminates read-through transcripts before they reach the IS 200 ORF. The terminator is functional in both directions and may terminate >80% of transcripts. Another control operates at the translational level: transposase synthesis is inhibited by occlusion of the ribosome-binding site (RBS) of the IS 200 ORF. The RBS (5'-AGGGG-3') is occluded by formation of a mRNA stem-loop **structure** whose 3' end is located only 3 nt upstream of the start codon. This mechanism **reduces** transposase synthesis approximately 10-fold. Primer extension experiments with AMV reverse transcriptase have provided evidence that this stem-loop **RNA structure** is actually formed. Tight repression of transposase synthesis, achieved through synergistic mechanisms of negative control, may explain the unusually low transposition frequency of IS 200.

4/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10341275 99350009 PMID: 10423146
The encapsidation signal of hepatitis B virus facilitates preC AUG recognition resulting in inefficient translation of the downstream genes.
Hwang W L; Su T S
Institute of Microbiology & Immunology, National Yang-Ming University, Taipei, Taiwan, Republic of China.
Journal of general virology (ENGLAND) Jul 1999, 80 (Pt 7) p1769-76, ISSN 0022-1317 Journal Code: 0077340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hepatitis B virus (HBV) DNA **polymerase** (P) is translated from a bicistronic pregenomic **RNA** via a ribosomal leaky scanning mechanism. Another viral transcript, the preC **RNA**, differs from pregenomic **RNA** by the presence of some 30 nt at the 5' end that encompass the preC initiation codon. This **RNA** is used exclusively for expression of the precore protein which is a precursor of secreted HBeAg. Factors leading to inefficient translation of the P and C proteins from the preC **RNA** were explored using a genetic approach in transient transfection assays. Our data indicate that when translating the precore protein, the elongation arrest that occurs during targeting of nascent polypeptide chains to the endoplasmic reticulum interferes with the scanning of the 40S ribosomal subunits. Such interference seems to hinder initiation of the ribosomes at

the downstream genes. Furthermore, the presence of the preC initiator codon in the preC mRNA has resulted in a **reduction** in the number of scanning ribosomes reaching the C and P initiator codons compared with the case of pregenomic **RNA**. Finally, although the preC initiator codon is in a suboptimal context for translation initiation, an **RNA secondary structure** , the encapsidation signal, located downstream to the initiator codon is shown to enhance codon recognition, resulting in a depletion of the number of 40S ribosomal subunits available for scanning of the downstream AUG codons. This study demonstrates that the HBV encapsidation signal plays an additional role in facilitating recognition of the preC initiator codon.

4/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10226047 99197090 PMID: 10094955
Growth inhibition by a triple ribozyme targeted to repetitive B2 transcripts.
Crone T M; Schalles S L; Benedict C M; Pan W; Ren L; Loy S E; Isom H; Clawson G A
Departments of Pathology, The Cell and Molecular Biology Program, The Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA, USA.
Hepatology (Baltimore, Md.) (UNITED STATES) Apr 1999, 29 (4) p1114-23, ISSN 0270-9139 Journal Code: 8302946
Contract/Grant No.: CA21141; CA; NCI; CA23931; CA; NCI; CA40145; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The B2 family represents a group of short repetitive sequences that are found throughout the rodent genome and are analogous to the human Alu sequences. Certain B2 subfamilies are transcribed by **RNA polymerase** III (pol III), and this transcription is in part controlled by the retinoblastoma protein. In addition to their putative role in retrotranspositional events, these actively transcribed B2 RNAs show a predicted highly stable **secondary structure**. Although B2 transcripts are normally confined to the nucleus, they demonstrate altered compartmentation after carcinogen treatment, in cancers, and in immortalized and/or transformed cell lines, the significance of which is unclear. Because modulation of B2 transcripts did not seem feasible with an antisense approach, we designed a triple ribozyme (TRz) construct to down-regulate B2 transcripts. The B2-targeted TRz undergoes efficient self-cleavage, resulting in liberation of the internal hammerhead Rz, which we targeted to a single-stranded region of the consensus B2 sequence. The liberated internal targeted Rz was 20 times more active than the corresponding double-G mutant construct that could not undergo self-cleavage, and 5 times more active than the same Rz flanked by nonspecific vector sequences. The B2-targeted TRz was used to develop stable transfectant clones from an SV40-immortalized hepatocyte cell line. These transfectant clones all showed variably **reduced** growth rates, accompanied by significant **reductions** in both cytoplasmic and nuclear B2 **RNA** levels: linear regression analyses showed that their growth rates were directly related to residual cytoplasmic B2 levels. Reverse-transcription **polymerase** chain reaction (RT-PCR) analyses documented efficient self-liberation of the internal targeted Rz in vivo, and showed that the relative cytoplasmic expression levels generally paralleled the magnitude of the decrease in B2 transcripts. The RT-PCR analyses further demonstrated that up to 20% of the Rz was located in the nucleus, which presumably reflects competition between autocatalytic processing and nucleocytoplasmic transport of the initial TRz transcript.

4/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10201292 99174053 PMID: 10074206
R region sequences in the long terminal repeat of a murine retrovirus specifically increase expression of unspliced RNAs.
Trubetskoy A M; Okenquist S A; Lenz J
Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York 10461, USA.
Journal of virology (UNITED STATES) Apr 1999, 73 (4) p3477-83, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA44822; CA; NCI; CA57337; CA; NCI; GM7288; GM; NIGMS ; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A stem-loop **structure** at the 5' end of the R region of the long terminal repeat (LTR) of the murine leukemia virus SL3 and other type C mammalian retroviruses is important for maximum levels of expression of a reporter gene under the control of the viral LTR. This element, termed the R region stem-loop (RSL), has a small effect on transcriptional initiation and no effect on **RNA polymerase** processivity. Its major effect is on posttranscriptional processing of LTR-driven transcripts. Here we tested whether the RSL affected the production of RNAs from a full-length SL3 genome. Mutation of the RSL in the 5' LTR of SL3 **reduced** the cytoplasmic levels of full-length viral transcripts but not those of spliced, env mRNA transcripts. Thus, the RSL specifically affected the cytoplasmic levels of the unspliced viral **RNA** . To test further whether the effect was specific for unspliced transcripts, a system was devised in which the expression of a reporter gene under the control of the viral LTR was tested in the presence or absence of an intron. Mutation of the RSL resulted in only about a twofold decline in the level of reporter gene expression when the transcripts contained an intron. However, when the intron was removed, mutation of the RSL **reduced** expression of the reporter gene about 10- to 60-fold in various cell lines. The **secondary structure** of the RSL was essential for its activity on the intronless transcript. Thus, the RSL appears to be important for the cytoplasmic accumulation of unspliced viral **RNA** and unspliced **RNA** from chimeric transcription units under the control of the viral LTR.

4/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10101641 99102563 PMID: 9847310
A hairpin **structure** in the R region of the human immunodeficiency virus type 1 **RNA** genome is instrumental in polyadenylation site selection.
Das A T; Klaver B; Berkhout B
Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.
Journal of virology (UNITED STATES) Jan 1999, 73 (1) p81-91, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Some retroviruses with an extended repeat (R) region encode the polyadenylation signal within the R region such that this signal is present at both the 5' and 3' ends of the viral transcript. This necessitates differential regulation to either repress recognition of the 5' polyadenylation signal or enhance usage of the 3' signal. The human

immunodeficiency virus type 1 (HIV-1) genome encodes an inherently efficient polyadenylation signal within the 97-nucleotide R region. Polyadenylation at the 5' HIV-1 polyadenylation site is inhibited by downstream splicing signals, and usage of the 3' polyadenylation site is triggered by an upstream enhancer element. In this paper, we demonstrate that this on-off switch of the HIV-1 polyadenylation signal is controlled by a **secondary RNA structure** that occludes part of the AAUAAA hexamer motif, which we have termed the polyA hairpin. Opening the 5' hairpin by mutation triggered premature polyadenylation and caused **reduced** synthesis of viral **RNA**, indicating that the **RNA structure** plays a pivotal role in repression of the 5' polyadenylation site. Apparently, the same hairpin **structure** does not interfere with efficient usage of the 3' polyadenylation site, which may be due to the presence of the upstream enhancer element. However, when the 3' hairpin was further stabilized by mutation, we measured a complete loss of 3' polyadenylation. Thus, the thermodynamic stability of the polyA hairpin is delicately balanced to allow nearly complete repression of the 5' site yet efficient activation of the 3' site. This is the first report of regulated polyadenylation that is mediated by **RNA secondary structure** . A similar hairpin motif that occludes the polyadenylation signal can be proposed for other lentiviruses and members of the spumaretroviruses, suggesting that this represents a more general gene expression strategy of complex retroviruses.

4/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09984101 98415727 PMID: 9744478

Dihydropteridine **reductase** deficiency: physical **structure** of the QDPR gene, identification of two new mutations and genotype-phenotype correlations.

Dianzani I; de Sanctis L; Smooker P M; Gough T J; Alliaudi C; Brusco A; Spada M; Blau N; Dobos M; Zhang H P; Yang N; Ponzone A; Armarego W L; Cotton R G

Dipartimento di Scienze Pediatriche e dell' Adolescenza, Universita degli Studi di Torino, Italy. dianzani@pediatria.unito.it

Human mutation (UNITED STATES) 1998, 12 (4) p267-73, ISSN 1059-7794 Journal Code: 9215429

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Dihydropteridine **reductase** (DHPR) is an enzyme involved in recycling of tetrahydrobiopterin (BH4), the cofactor of the aromatic amino acid hydroxylases. Its deficiency is characterized by hyperphenylalaninemia due to the **secondary** defect of phenylalanine hydroxylase and depletion of the neurotransmitters dopamine and serotonin, whose syntheses are controlled by tryptophan and tyrosine hydroxylases. The DHPR cDNA has been cloned and mapped on 4p15.3. In the present study we report the genomic **structure** of the DHPR gene (QDPR). This gene includes seven exons within a range of 84-564 bp; the corresponding introns are flanked by canonic splice junctions. We also present a panel of PCR primers complementary to intronic sequences that greatly facilitates amplification of the gene and provides a genomic DNA approach for mutation detection. We have used this approach to study six patients with DHPR deficiency. Four known mutations (G23D, H158Y, IVS5G+ 1A, R221X) and two new mutations (Y150C and G218ins9bp) were found. The Y150C mutation was found in compound heterozygosity with G23D, a mutation always associated with a severe phenotype in homozygous patients. This patient has an intermediate phenotype (good response to monotherapy with BH4). The mutant enzyme for Y150C was expressed in an E. coli system. Comparison of its kinetic parameters with those of the G23D mutant enzyme showed that it is not as effective as the wild-type enzyme, but is more active than the G23D mutant.

This patient's intermediate phenotype is thus due to the mild DHPR mutation Y150C. Correlations between genotypes and phenotypes were also found for the other mutations.

4/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09957772 98395116 PMID: 9727013
A novel mutation in the switch 3 region of Gsalpha in a patient with Albright hereditary osteodystrophy impairs GDP binding and receptor activation.
Warner D R; Weng G; Yu S; Matalon R; Weinstein L S
Membrane Biochemistry Section, Laboratory of Molecular and Cellular Neurobiology, NINDS, National Institutes of Health, Bethesda, Maryland 20892, USA. dwarner@helix.nih.gov
Journal of biological chemistry (UNITED STATES) Sep 11 1998, 273 (37) p23976-83, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Albright hereditary osteodystrophy (AHO), a disorder characterized by skeletal abnormalities and obesity, is associated with heterozygous inactivating mutations in the gene for Gsalpha. A novel Gsalpha mutation encoding the substitution of tryptophan for a nonconserved arginine within the switch 3 region (Gsalpha R258W) was identified in an AHO patient. Although reverse transcription-**polymerase** chain reaction studies demonstrated that mRNA expression from wild type and mutant alleles was similar, Gsalpha expression in erythrocyte membranes from the affected patient was **reduced** by 50%. A Gsalpha R258W cDNA, as well as one with arginine replaced by alanine (Gsalpha R258A), was generated, and the biochemical properties of in vitro transcription/translation products were examined. When reconstituted with cyc- membranes, both mutant proteins were able to stimulate adenylyl cyclase normally in the presence of guanosine-5'-O-(3-thiotriphosphate) (GTPgammaS) but had decreased ability in the presence of isoproterenol or AlF4- (a mixture of 10 microM AlCl3 and 10 mM NaF). The ability of each mutant to bind and be activated by GTPgammaS or AlF4- was assessed by trypsin protection assays. Both mutants were protected normally by GTPgammaS but showed **reduced** protection in the presence of AlF4-. The addition of excess GDP (2 mM) was able to rescue the ability of AlF4- to protect the mutants, suggesting that they might have **reduced** affinity for GDP. A Gsalpha R258A mutant purified from Escherichia coli had decreased affinity for GDP and an apparent rate of GDP release that was 10-fold greater than that of wild type Gsalpha. Sucrose density gradient analysis demonstrated that both Gsalpha R258W and Gsalpha R258A were thermolabile at higher temperatures and that denaturation of both mutants was prevented by the presence of 0.1 mM GTPgammaS or 2 mM GDP. The crystal **structure** of Gsalpha demonstrates that Arg258 interacts with a conserved residue in the helical domain (Gln170). Arg258 substitutions would be predicted to open the cleft between the GTPase and helical domains, allowing for increased GDP release in the inactive state, resulting in enhanced thermolability and **reduced** AlF4--induced adenylyl cyclase stimulation and trypsin protection, since activation by AlF4- requires bound GDP.

4/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09936244 98357527 PMID: 9694154
Efficient ex vivo inhibition of perforin and Fas ligand expression by chimeric tRNA-hammerhead ribozymes.
Du Z; Ricordi C; Inverardi L; Podack E; Pastori R L

Diabetes Research Institute, University of Miami School of Medicine, FL 33136, USA.
Human gene therapy (UNITED STATES) Jul 20 1998, 9 (11) p1551-60, ISSN 1043-0342 Journal Code: 9008950
Contract/Grant No.: DK-25802; DK; NIDDK; DK-50710; DK; NIDDK
Comment in Hum Gene Ther. 1998 Jul 20;9(11) 1528-9; Comment in PMID 9694151
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Graft-versus-host disease (GVHD) is a feared complication of allogeneic bone marrow transplantation. Research in rodent models has linked perforin and Fas ligand (FasL), two components of independent lytic pathways, with the induction of GVHD. In this study we characterized two hammerhead ribozymes that cleave their target perforin and Fas ligand RNAs with high efficiency in CTLL-2 cells. The perforin and Fas ligand ribozymes were expressed from a tRNA-directed **RNA polymerase** III promoter that was inserted in an episomal multicopy plasmid derived from papilloma virus. Chimeric anti-perforin and anti-FasL tRNA-ribozymes had sequences engineered in order to have specific **secondary structure** effects. These sequence modifications allow the formation of a 5' --> 3' stem **structure** and also place the ribozyme in a flexible bulge region that keeps the ribozyme separated from the tRNA domain. Northern and RT in situ PCR analyses showed high levels of transcription and efficient transportation to the cytoplasm. The expression of perforin and FasL in CTLL-2 cells was significantly **reduced** as assessed by **RNA** and protein analyses.

4/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09906398 98333576 PMID: 9668979
High-temperature, nonradioactive primer extension assay for determination of a transcription-initiation site.
Yamada M; Izu H; Nitta T; Kurihara K; Sakurai T
Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan.
BioTechniques (UNITED STATES) Jul 1998, 25 (1) p72-4, 76, 78, ISSN 0736-6205 Journal Code: 8306785
Document type: Technical Report
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have developed a simple and safe method for the determination of a transcription-initiation site. In this method, reverse transcriptase of the avian myeloblastosis virus or rTth DNA **polymerase** from Thermus thermopilus was used with a fluorescein isothiocyanate (FITC)-labeled primer. The primer-extension reaction can be performed at a high temperature, which **reduces** the hindering effect of the **secondary structure** in **RNA**, and can omit the annealing step between **RNA** and the primer. Almost all steps can be done in one tube. This procedure can provide reliable and reproducible data when compared with the conventional procedure at low temperature. Moreover, the sequencing ladder that is required for determining the position of extended products can be obtained with the same FITC-labeled primer.

4/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09747327 98184861 PMID: 9516454
Insertions into the beta3-beta4 hairpin loop of HIV-1 reverse

transcriptase reveal a role for fingers subdomain in processive polymerization.

Kew Y; Olsen L R; Japour A J; Prasad V R

Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, USA.

Journal of biological chemistry (UNITED STATES) Mar 27 1998, 273 (13) p7529-37, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: AI-30861; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) displays a characteristic poor processivity during DNA polymerization. Structural elements of RT that determine processivity are poorly understood. The three-dimensional **structure** of HIV-1 RT, which assumes a hand-like **structure** , shows that the fingers, palm, and thumb subdomains form the template-binding cleft and may be involved in determining the degree of processivity. To assess the influence of fingers subdomain of HIV-1 RT in **polymerase** processivity, two insertions were engineered in the beta3-beta4 hairpin of HIV-1NL4-3 RT. The recombinant mutant RTs, named FE20 and FE103, displayed wild type or near wild type levels of **RNA**-dependent DNA **polymerase** activity on all templates tested and wild type or near wild type-like sensitivities to dideoxy-NTPs. When **polymerase** activities were measured under conditions that allow a single cycle of DNA polymerization, both of the mutants displayed 25-30% greater processivity than wild type enzyme. Homology modeling the three-dimensional structures of wild type HIV-1NL4-3 RT and its finger insertion mutants revealed that the extended loop between the beta3 and beta4 strands protrudes into the cleft, **reducing** the distance between the fingers and thumb subdomains to approximately 12 A. Analysis of the models for the mutants suggests an extensive interaction between the protein and template-primer, which may **reduce** the degree of superstructure in the template-primer. Our data suggest that the beta3-beta4 hairpin of fingers subdomain is an important determinant of processive polymerization by HIV-1 RT.

4/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09636883 98034460 PMID: 9368099

Display of disparate transcription phenotype by the phosphorylation negative P protein mutants of vesicular stomatitis virus, Indiana serotype, expressed in E. coli and eucaryotic cells.

Mathur M; Das T; Chen J L; Chattopadhyay D; Banerjee A K

Department of Molecular Biology, Cleveland Clinic Foundation, OH 44195, USA.

Gene expression (UNITED STATES) 1997, 6 (5) p275-86, ISSN 1052-2166 Journal Code: 9200651

Contract/Grant No.: AI26585; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The phosphoprotein (P) of vesicular stomatitis virus (VSV) is a subunit of the **RNA polymerase** (L) that transcribes the negative strand genome **RNA** into mRNAs both in vitro and in vivo. We have recently shown that the P protein of VSV, New Jersey serotype (PNJ), expressed in E. coli, is biologically inactive unless phosphorylated at specific serine residues by cellular casein kinase II (CKII). In the present work, we are studying the role of phosphorylation in the activation of the P protein of Indiana serotype (PIND), which is highly nonhomologous in amino acid sequence yet structurally similar to its New Jersey counterpart. Despite

the fact that E. coli-expressed PIND required phosphorylation by CKII for activation, the phosphorylation negative P protein mutants generated by altering the phosphate acceptors S and T to alanine, surprisingly, showed transcription activity similar to wild-type in vitro. Alteration of S and T residues to phenylalanine, similarly, supported substantial transcription activity (approx. 60% of wild-type), whereas substitution with arginine residue abrogated transcription (approx. 5% of wild-type). In contrast, the same mutants, when expressed in eucaryotic cells, exhibited greatly **reduced** transcription activity in vitro. This disparate display of transcription phenotype by the PIND mutants expressed in bacteria and eucaryotic cells suggests that these mutants are unique in assuming different **secondary structure** or conformation when synthesized in two different cellular milieu. The findings that, unless phosphorylated by CKII, the bacterially expressed unphosphorylated (P0) form of PIND, as well as the phosphorylation negative mutants expressed in eucaryotic cells, demonstrates transcription negative phenotype indicate that, like PNJ, phosphorylation of PIND is essential for its activity.

4/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09633044 98054271 PMID: 9391063

Escherichia coli **RNA polymerase** terminates transcription efficiently at rho-independent terminators on single-stranded DNA templates.

Uptain S M; Chamberlin M J

Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 9 1997, 94 (25) p13548-53, ISSN 0027-8424
Journal Code: 7505876

Contract/Grant No.: GM 07232; GM; NIGMS; GM 12010; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Several models have been proposed for the mechanism of transcript termination by Escherichia coli **RNA polymerase** at rho-independent terminators. Yager and von Hippel (Yager, T. D. & von Hippel, P. H. (1991) Biochemistry 30, 1097-118) postulated that the transcription complex is stabilized by enzyme-nucleic acid interactions and the favorable free energy of a 12-bp **RNA** -DNA hybrid but is destabilized by the free energy required to maintain an extended transcription bubble. Termination, by their model, is viewed simply as displacement of the **RNA** transcript from the hybrid helix by reformation of the DNA helix. We have proposed an alternative model where the **RNA** transcript is stably bound to **RNA polymerase** primarily through interactions with two single-strand specific **RNA** -binding sites; termination is triggered by formation of an **RNA** hairpin that **reduces** binding of the **RNA** to one **RNA** -binding site and, ultimately, leads to its ejection from the complex. To distinguish between these models, we have tested whether E. coli **RNA polymerase** can terminate transcription at rho-independent terminators on single-stranded DNA. **RNA polymerase** cannot form a transcription bubble on these templates; thus, the Yager-von Hippel model predicts that intrinsic termination will not occur. We find that transcript elongation on single-stranded DNA templates is hindered somewhat by DNA **secondary structure**. However, E. coli **RNA polymerase** efficiently terminates and releases transcripts at several rho-independent terminators on such templates at the same positions as termination occurs on duplex DNAs. Therefore, neither the nontranscribed DNA strand nor the transcription bubble is essential for rho-independent termination by E. coli **RNA polymerase**.

4/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09620423 98038746 PMID: 9372909
Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcription factor IIB (TFIIB): identification of an N-terminal mutant that stabilizes TATA-binding protein-TFIIB-DNA complexes.
Bangur C S; Pardee T S; Ponticelli A S
Department of Biochemistry and Center for Advanced Molecular Biology and Immunology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, 14214-3000, USA.
Molecular and cellular biology (UNITED STATES) Dec 1997, 17 (12) p6784-93, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: GM51124; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The general transcription factor IIB (TFIIB) plays an essential role in transcription of protein-coding genes by **RNA polymerase** II. We have used site-directed mutagenesis to assess the role of conserved amino acids in several important regions of yeast TFIIB. These include residues in the highly conserved amino-terminal region and basic residues in the D1 and E1 core domain alpha-helices. Acidic substitutions of residues K190 (D1) and K201 (E1) resulted in growth impairments in vivo, **reduced** basal transcriptional activity in vitro, and an inability to form stable TFIIB-TATA-binding protein-DNA (DB) complexes. Significantly, these mutants retained the ability to respond to acidic activators in vivo and to the Gal4-VP16 activator in vitro, supporting the view that these basic residues play a role in basal transcription. In addition, 14 single-amino-acid substitutions were introduced in the conserved amino-terminal region. Three of these mutants, the L50D, R64E, and R78L mutants, displayed altered growth properties in vivo and were compromised for supporting transcription in vitro. The L50D mutant was impaired for **RNA polymerase** II interaction, while the R64E mutant exhibited altered transcription start site selection both in vitro and in vivo and, surprisingly, was more active than the wild type in the formation of stable DB complexes. These results support the view that the amino-terminal domain is involved in the direct interaction between yeast TFIIB and **RNA polymerase** II and suggest that this domain may interact with DNA and/or modulate the formation of a DB complex.

4/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09594516 98006388 PMID: 9348165
Impact of dengue virus infection and its control.
Igarashi A
Department of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki City, Japan.
FEMS immunology and medical microbiology (NETHERLANDS) Aug 1997, 18 (4) p291-300, ISSN 0928-8244 Journal Code: 9315554
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Dengue virus infection has been counted among emerging and re-emerging diseases because of (1) the increasing number of patients, (2) the expansion of epidemic areas, and (3) the appearance of severe clinical manifestation of dengue hemorrhagic fever (DHF)/dengue shock syndrome

(DSS), which is often fatal if not properly treated. In the meantime, there are no effective dengue control measures: a dengue vaccine is still under development and vector control does not provide a long-lasting effect. In order to obtain direct evidence for the virulent virus theory concerning the pathogenesis of DHF/DSS, type 2 dengue virus strains isolated from patients with different clinical severities in the same epidemic area in northeast Thailand, during the same season, were comparatively sequenced. The result revealed a DF strain specific amino acid substitution from I to R in the PrM, and a DSS strain specific amino acid substitution from D to G in the NS1 gene regions, which could significantly alter the nature of these proteins. Moreover, DF strain specific nucleotide substitutions in the 3' noncoding region were predicted to alter its **secondary structure** . These amino acid and nucleotide substitutions in other strains isolated in different epidemic areas during other seasons, together with their biological significance, remain to be confirmed. In order to innovate dengue vector control, field tests were carried out in dengue epidemic areas in Vietnam to examine the efficacy of Olyset Net screen, which is a wide-mesh net made of polyethylene thread impregnated with permethrin. The results show that Olyset Net (1) **reduced** the number of principal dengue vector species, Aedes aegypti, (2) interrupted the silent transmission of dengue viruses and (3) was highly appreciated by the local people as a convenient and comfortable vector control method. This encouraging evaluation of the Olyset Net screen should be confirmed further by other tests under different settings.

4/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09590017 98026218 PMID: 9380524
Betaine improves the PCR amplification of GC-rich DNA sequences.
Henke W; Herdel K; Jung K; Schnorr D; Loening S A
Research Division of the Department of Urology, University Hospital Charite, Humboldt University, D-10098 Berlin, Germany. henke@rz.charite.hu-berlin.de
Nucleic acids research (ENGLAND) Oct 1 1997, 25 (19) p3957-8, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Betaine improves the co-amplification of the two alternatively spliced variants of the prostate-specific membrane antigen mRNA as well as the amplification of the coding cDNA region of c-jun. It is suggested that betaine improves the amplification of these genes by **reducing** the formation of **secondary structure** caused by GC-rich regions and, therefore, may be generally applicable to ameliorate the amplification of GC-rich DNA sequences.

4/3,AB/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09504415 97407963 PMID: 9261187
Translation of an uncapped mRNA involves scanning.
Gunnery S; Maivali U; Mathews M B
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.
Journal of biological chemistry (UNITED STATES) Aug 22 1997, 272 (34) p21642-6, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: AI31822; AI; NIAID; CA13106; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

tat, an essential gene of human immunodeficiency virus, when placed under the control of the **RNA polymerase** III promoter from the adenovirus VA RNA1 gene, is transcribed into an uncapped and nonpolyadenylated mRNA. This VA-Tat **RNA** is translated to produce functional Tat protein in transfected mammalian cells (Gunnery, S., and Mathews, M. B. (1995) Mol. Cell. Biol. 15, 3597-3607). The presence of an upstream open reading frame (ORF) in VA-Tat **RNA** is inhibitory to the translation of the Tat ORF, suggesting that the **RNA** is scanned during translation even though it is uncapped. Because the effect of the upstream ORF is relatively small (about 2-fold), we sought more definitive evidence of scanning by introducing **secondary** structures of varying stabilities into the 5'-untranslated region of VA-Tat **RNA**. The results of transfection experiments showed that highly stable **secondary structure** was inhibitory to Tat synthesis, whereas structures of lower stability were not inhibitory, confirming that uncapped mRNA is subject to scanning. Furthermore, translation of the downstream ORF was **reduced** but not eliminated by mutations that caused the upstream ORF to overlap the Tat ORF. Extending the overlap of the two ORFs further decreased the translation of the downstream ORF. This observation implies that ribosomes reinitiate after termination, possibly after migrating in a 3' to 5' direction through the overlap region of the mRNA. Similar results were obtained with a capped **polymerase** II transcript, indicating that the translation of **polymerase** II and **polymerase** III transcripts occurs through similar mechanisms.

4/3,AB/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09428494 97317115 PMID: 9211980
Variable region V1 of Saccharomyces cerevisiae 18S rRNA participates in biogenesis and function of the small ribosomal subunit.
van Nues R W; Venema J; Planta R J; Raue H A
Department of Biochemistry and Molecular Biology, IMBW, BioCentrum Amsterdam, Vrije Universiteit, de Boelelaan 1083, 1081 HV, Amsterdam, The Netherlands.
Chromosoma (GERMANY) Jun 1997, 105 (7-8) p523-31, ISSN 0009-5915 Journal Code: 2985138R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The role of helix 6, which forms the major portion of the most 5'-located expansion segment of Saccharomyces cerevisiae 18S rRNA, was studied by in vivo mutational analysis. Mutations that increased the size of the helical part and/or the loop, even to a relatively small extent, abolished 18S rRNA formation almost completely. Concomitantly, 35S pre-rRNA and an abnormal 23S precursor species accumulated. rDNA units containing these mutations did not support cell growth. A deletion removing helix 6 almost completely, on the other hand, had a much less severe effect on the formation of 18S rRNA, and cells expressing only the mutant rRNA remained able to grow, albeit at a much **reduced** rate. Disruption of the apical A.U base pair by a single point mutation did not cause a noticeable **reduction** in the level of 18S rRNA but did result in a twofold lower growth rate of the cells. This effect could not be reversed by introduction of a second point mutation that restores base pairing. We conclude that both the primary and the **secondary structure** of helix 6 play an important role in the formation and the biological function of the 40S subunit.

4/3,AB/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09388074 97286545 PMID: 9141692

The temperature sensitivity of Bacillus subtilis DB1005 is due to insufficient activity, rather than insufficient concentration, of the mutant delta A factor.

Chang B Y; Liao C T; Wen Y D; Wang W H
Agricultural Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan, Republic of China. bychang@mail.nchu.edu.rw

Microbiology (Reading, England) (ENGLAND) Apr 1997, 143 (Pt 4) p1299-308, ISSN 1350-0872 Journal Code: 9430468
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The delta A factor of Bacillus subtilis DB1005 contains two amino acid substitutions (I198A and I202A) in the promoter-10 binding region. It has been confirmed that this delta factor is responsible for the temperature sensitivity of B. subtilis DB1005. An investigation was conducted into how the mutant delta A could cause temperature-sensitive (Ts) cell growth by analysing its structural stability, cellular concentration and transcriptional activity. The mutant delta A was unstable even at the permissive temperature of 37 degrees C (t1/2 59 min), whereas the wild-type counterpart was fairly stable under the same conditions (t1/2 > 600 min). However, neither wild-type delta A nor mutant delta A was stable at 49 degrees C (t1/2 34 min and 23 min, respectively). Analyses of the rates of delta A synthesis revealed that B. subtilis DB1005 was able to compensate for unstable delta A by elevating the level of delta A at 37 degrees C but not at 49 degrees C. Moreover, overexpression of the mutant delta A at 49 degrees C could not suppress the Ts phenotype of B. subtilis DB1005. This indicates that the temperature sensitivity of B. subtilis DB1005 is not due to insufficient delta A concentration in the cell. The greater decline of an already **reduced** activity of the mutant delta A at 49 degrees C suggests that the temperature sensitivity of B. subtilis DB1005 is instead the result of a very low activity of delta A; probably below a critical level necessary for cell growth.

4/3,AB/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09381845 97293181 PMID: 9149234

Secondary structure of the nascent 7S L **RNA** mediates efficient transcription by **RNA polymerase** III.

Emde G; Frontzek A; Benecke B J
Department of Biochemistry, Ruhr-University, Bochum, Germany.

RNA (New York, N.Y.) (UNITED STATES) May 1997, 3 (5) p538-49, ISSN 1355-8382 Journal Code: 9509184
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A structural motif at the 5' end of human 7S L (srp) **RNA** that is recognized specifically by cellular proteins has been identified as an efficient activator of **RNA polymerase** (pol) III transcription in vivo and in vitro. Mutations affecting three double-stranded regions or a tetranucleotide bulge of this **RNA** motif result in strongly **reduced** expression rates. However, effective suppression is achieved by compensatory mutations restoring **RNA** sequence complementarity. This activation of transcription is also observed in the context of another pol III promoter and is position-dependent. The effects observed are reminiscent of the Tat-TAR trans-activation of the human immunodeficiency virus and attribute a novel function to the **structure** of cellular small stable **RNA**.

4/3,AB/44 (Item 44 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09203877 97100225 PMID: 8944786
Contribution of individual tryptophan residues to the **structure** and activity of theta-toxin (perfringolysin O), a cholesterol-binding cytolysin.
Sekino-Suzuki N; Nakamura M; Mitsui K I; Ohno-Iwashita Y
Department of Enzyme Biochemistry, Tokyo Metropolitan Institute of Gerontology, Japan.
European journal of biochemistry / FEBS (GERMANY) Nov 1 1996, 241 (3) p941-7, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
theta-Toxin (perfringolysin O), secreted by Clostridium perfringens, shares with other known thiol-activated toxins a conserved undecapeptide, ECTGLAWEWWR, located in the C-terminal region of the protein and containing the unique cysteine of the molecule. Single and double amino acid substitutions were created in the theta-toxin molecule to investigate the role of individual tryptophan residues in the lytic activity of theta-toxin. Wild-type and mutant theta-toxins were overproduced in Escherichia coli by means of a T7 **RNA polymerase**/promoter system and purified. The relative hemolytic activities of four mutant toxins, each with a Trp to Phe substitution outside the common Cys-containing region, were more than 60% that of wild-type theta-toxin. In contrast, mutant toxins with Phe replacements within the Cys-containing region (at Trp436, Trp438 or Trp439) showed significantly **reduced** hemolytic and erythrocyte-membrane-binding activities. The largest **reduction** in binding affinity, more than 100-fold, was observed for Trp438 mutant toxins. However, the mutants retain binding specificity for cholesterol and the ability to form arc-shaped and ring-shaped structures on membranes. These results indicate that the low hemolytic activities of these mutant toxins can be ascribed, at least in part, to **reduced** binding activities. With respect to protease susceptibility and far-ultraviolet circular-dichroism spectra, only the W436-->F mutant toxin, showed any considerable difference from wild-type toxin in **secondary** or higher-order structures, indicating that Trp436 is essential for maintenance of toxin **structure**.

4/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09195650 97116021 PMID: 8957113
Isolation and expression of a cDNA clone encoding an Alternaria alternata Alt a 1 subunit.
De Vouge M W; Thaker A J; Curran I H; Zhang L; Muradia G; Rode H; Vijay H M
Life Sciences Division, Bureau of Drug Research, Health Canada, Ottawa.
International archives of allergy and immunology (SWITZERLAND) Dec 1996, 111 (4) p385-95, ISSN 1018-2438 Journal Code: 9211652
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Alternaria alternata is recognized as an important source of fungal aeroallergens. Alt a 1, the major allergen of this mold, is a dimer of disulfide-linked subunits that migrate in SDS-PAGE under **reducing** conditions at apparent M(r)s of 14,500 and 16,000. IgE antibodies to this protein are present in the sera of >90% of A. alternata-sensitive individuals. Previous studies from this laboratory showed that the N-termini twenty amino acids of the purified subunits are nearly identical. We now report the isolation of clones from an A. alternata (strain 34-016)

cDNA library constructed in lambda(gt)11, using rabbit IgG antiserum against partially purified Alt a 1. One of nineteen clones selected from screens totalling 305,000 pfu (rb51) was sequenced, and determined to harbor an insert of 660 bp. An in-frame open reading frame within the cloned insert encodes a peptide of M(r) 16,960 that bears no significant homology to known allergens or proteins. The size of the rb51 transcript was determined to be approximately 0.7 kb by Northern analysis of A. alternata total **RNA**. The largely hydrophobic N-terminal region of the peptide contains an alpha-helical domain and other features characteristic of membrane targeting or secretory signals. The peptide sequence downstream of this region matches previously sequenced Alt a 1 N-terminal from two independent sources at 17 of 20, and 24 of 26 positions. Recombinant Alt a 1 expressed as a secreted protein in Pichia pastoris exists as a dimer in conditioned medium, as shown by immunoblotting under nonreducing conditions. Recombinant Alt a 1, like the natural allergen in A. alternata extracts, is also reactive with serum IgE from A. alternata-sensitive individuals.

4/3,AB/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09125274 97016181 PMID: 8862818
Direct amplification and cloning of up to 5-kb lentivirus genomes from serum.
Holterman L; Mullins J I; Haaijman J J; Heeney J L
Biomedical Primate Research Centre, Rijswijk, The Netherlands.
BioTechniques (UNITED STATES) Aug 1996, 21 (2) p312-9, ISSN 0736-6205 Journal Code: 8306785
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To produce large cDNA strands from biological samples containing limited numbers of template molecules, it may be necessary to minimize both nonspecific primer attachment in first-strand synthesis and **secondary structure** in **RNA** molecules. Failure to do so could result in the accumulation of shortened cDNA strands and therefore may **reduce** the yield of large cDNA molecules, sometimes below detection level. We show that 5.0-kb cDNA fragments can be generated from simian immunodeficiency virus **RNA** in a specific reverse transcription (RT)-PCR by increasing the stringency of the primer-annealing conditions, followed by the elimination of excess free primer. Since this method utilizes a relatively long primer in the first-strand cDNA synthesis, it is possible to heat-denature the nonspecific **RNA**/primer complexes and **RNA secondary structure** without dissociating the primer from the specific template. In contrast to classic RT assays, in which an excess of primer is annealed to denatured **RNA** just prior to and during reverse transcription at relative low temperatures (37 degrees-42 degrees C), this method eliminates false priming. To optimize the yield and fidelity of full-length cDNA molecules, two PCR amplifications are first performed using both Taq and Pfu **polymerase** , followed by Pfu alone in the second amplification.

4/3,AB/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09029225 96400143 PMID: 8806519
Epstein-Barr virus single-stranded DNA-binding protein: purification, characterization, and action on DNA synthesis by the viral DNA **polymerase**.
Tsurumi T; Kobayashi A; Tamai K; Yamada H; Daikoku T; Yamashita Y; Nishiyama Y

Laboratory of Virology, Nagoya University School of Medicine, Japan.
Virology (UNITED STATES) Aug 15 1996, 222 (2) p352-64, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Epstein-Barr virus (EBV) BALF2 gene product is one of the essential components in the lytic phase of the EBV DNA replication. The BALF2 protein was purified to near homogeneity from the nuclear extract of B95-8 cells with virus productive cycle induced by chemical agents. SDS-polyacrylamide gel electrophoresis showed the presence of a single polypeptide with a molecular weight of 130 K, which was identified as BALF2 protein by Western immunoblot analysis. On Superose 6 HR 10/30 gel filtration the BALF2 protein eluted at a position corresponding to an apparent molecular mass of approximately 128 K, indicating that the BALF2 protein behaves as a monomer in solution. The purified BALF2 protein bound to single-stranded DNA preferentially over double-stranded DNA or single-stranded **RNA**. Replication of singly primed M13 single-stranded DNA by the EBV DNA **polymerase** complex in the absence of the BALF2 protein exhibited a highly processive mode of replication and generated full length products in addition to some bands of pausing sites. Although the addition of the BALF2 protein did not affect the replication rate, the average chain length of the replication products was slightly increased with eliminating bands of pausing sites. Similar effects were observed with the reconstituted **polymerase** complex composed of the BALF5 and BMRF1 Pol subunits. On the other hand, in the absence of the BALF2 protein, the BALF5 Pol catalytic subunit alone extended the primer slightly and paused at specific sites on M13 ssDNA template where stable **secondary structure** is predicted. However, addition of the BALF2 protein, in contrast to the case of herpes simplex virus ICP8 which does not affect the overall distribution of length of the replication products synthesized by the HSV Pol catalytic subunit (Gottlieb et al., 1990, J. Virol. 64, 5976-5987), stimulated DNA synthesis and yielded a distribution of replication products with long lengths in addition to full length products. Although the BALF2 protein behaved as if it converts a low processive enzyme of the EBV Pol catalytic subunit to a highly processive form like the BMRF1 Pol accessory subunit, challenger DNA experiments revealed that the EBV Pol catalytic subunit is transferred to challenger DNA even in the presence of the BALF2 protein. It is therefore likely that the EBV BALF2 protein functions to melt out the regions of **secondary structure** on the single-stranded DNA template, thereby **reducing** and eliminating pausing of the EBV DNA **polymerase** at specific sites. These properties indicate that the EBV BALF2 protein acts as a single-stranded DNA-binding protein during lytic phase of EBV DNA replication.

4/3,AB/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08996789 96366792 PMID: 8770906
A disulfide bonding interaction role for cysteines in the extracellular domain of the thyrotropin-releasing hormone receptor.
Cook J V; McGregor A; Lee T; Milligan G; Eidne K A
Medical Research Council Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, Scotland, United Kingdom.
Endocrinology (UNITED STATES) Jul 1996, 137 (7) p2851-8, ISSN 0013-7227 Journal Code: 0375040
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The roles of disulfide and sulfhydryl groups in the specific binding of TRH to its receptor have been examined. In all TRH receptors (TRH-Rs)

isolated from different species so far, there are only two extracellular cysteine residues (Cys98 in the extracellular loop between transmembrane helices 2 and 3 and Cys179 in the extracellular loop between transmembrane helices 4 and 5) that are in positions homologous to cysteine residues in other G protein-coupled receptors. Another Cys (Cys100) is located in close proximity to Cys98 at the interface between the first extracellular loop and third transmembrane domain. To assess the role of these TRH-R Cys residues in disulfide bonding interactions, they were mutated to either Ser or Ala. Six mutant receptors (Cys98Ser, Cys98Ala, Cys179Ser, Cys179Ala, Cys100Ser, and Cys100Ala) were expressed in COS-1 cells and tested for their ability to bind TRH and to activate total inositol phosphate (IP) formation. TRH-R mutants Cys100Ser and Cys100Ala showed TRH binding affinities and IP activation similar to the wild-type (WT). In contrast, mutants Cys98Ser, Cys98Ala, Cys179Ser, and Cys179Ala showed no high affinity TRH binding. The potencies of Cys98Ala and Cys179Ala as measured by IP stimulation were decreased by four orders of magnitude when compared with WT. Cys98Ser potency decreased by five orders of magnitude, whereas Cys179Ser showed no IP production. Northern blotting confirmed expression of all the mutant TRH-Rs at the messenger **RNA** (mRNA) level. An epitope tag derived from the Haemophilus influenza hemagglutinin protein was incorporated at the NH2 termini of the TRH-R WT and TRH-R Cys mutants to allow the independent assessment of cell surface expression of receptor protein. TRH-R mutants that failed to show receptor binding (Cys98Ser, Cys98Ala, Cys179Ala) showed WT levels of cell surface receptor expression, indicating that loss of receptor binding in these mutants is not attributable to loss of receptor expression. In contrast, cell surface expression of Cys179Ser, which showed no ligand induced IP stimulation, could not be detected. Dithiothreitol, a disulfide bond **reducing** agent, and p-chloromercuribenzoic acid (p-CMB), a sulfhydryl blocking compound, **reduced** specific TRH binding in a dose-dependent manner. The inhibition of binding by dithiothreitol implies that the integrity of a disulfide bond is important for TRH binding to its receptor. The dramatic inhibition of TRH binding by p-CMB indicates that free sulfhydryl groups are also associated with the binding of the ligand to its receptor. This study presents evidence that a disulfide bond exists between Cys98 and Cys179 which is essential for maintaining the receptor in the correct conformation for ligand binding. Cys100 is not thought to have a disulfide bonding interaction role. Results obtained after chemical modification have shown that free sulfhydryl groups within the TRH-R may also have a role in ligand interactions.

4/3,AB/49 (Item 49 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08961740 96323149 PMID: 8709255
Cell proteins bind specifically to West Nile virus minus-strand 3' stem-loop **RNA**.
Shi P Y; Li W; Brinton M A
Department of Biology, Georgia State University, Atlanta 30303, USA.
Journal of virology (UNITED STATES) Sep 1996, 70 (9) p6278-87, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI 18382; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The first 96 nucleotides of the 5'noncoding region (NCR) of West Nile virus (WNV) genomic **RNA** were previously reported to form thermodynamically predicted stem-loop (SL) structures that are conserved among flaviviruses. The complementary minus-strand 3' NCR **RNA**, which is thought to function as a promoter for the synthesis of plus-strand **RNA**, forms a corresponding predicted SL **structure** . RNase probing of the WNV 3' minus-strand stem-loop **RNA** [WNV (-)3' SL

RNA] confirmed the existence of a terminal **secondary structure**. **RNA**-protein binding studies were performed with BHK S100 cytoplasmic extracts and in vitro-synthesized WNV (-)3' SL **RNA** as the probe. Three **RNA**-protein complexes (complexes 1,2, and 3) were detected by a gel mobility shift assay, and the specificity of the **RNA** -protein interactions was confirmed by gel mobility shift and UV-induced cross-linking competition assays. Four BHK cell proteins with molecular masses of 108, 60, 50, and 42 kDa were detected by UV-induced cross-linking to the WNV (-)3' SL **RNA**. A preliminary mapping study indicated that all four proteins bound to the first 75 nucleotides of the WNV 3' minus-strand **RNA**, the region that contains the terminal SL. A flavivirus resistance phenotype was previously shown to be inherited in mice as a single, autosomal dominant allele. The efficiencies of infection of resistant cells and susceptible cells are similar, but resistant cells (C3H/RV) produce less genomic **RNA** than congenic, susceptible cells (C3H/He). Three **RNA** -protein complexes and four UV-induced cross-linked cell proteins with mobilities identical to those detected in BHK cell extracts with the WNV (-)3' SL **RNA** were found in both C3H/RV and C3H/He cell extracts. However, the half-life of the C3H/RV complex 1 was three times longer than that of the C3H/He complex 1. It is possible that the increased binding activity of one of the resistant cell proteins for the flavivirus minus-strand **RNA** could result in a **reduced** synthesis of plus-strand **RNA** as observed with the flavivirus resistance phenotype.

4/3,AB/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08936878 96302247 PMID: 8755728
Human ferredoxin: overproduction in Escherichia coli, reconstitution in vitro, and spectroscopic studies of iron-sulfur cluster ligand cysteine-to-serine mutants.
Xia B; Cheng H; Bandarian V; Reed G H; Markley J L
Department of Biochemistry, University of Wisconsin-Madison 53706, USA.
Biochemistry (UNITED STATES) Jul 23 1996, 35 (29) p9488-95, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM 35752; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Human ferredoxin, the human equivalent of bovine adrenodoxin, is a small iron-sulfur protein with one [2Fe-2S] cluster. It functions, as do other vertebrate ferredoxins, to transfer electrons during the processes of steroid hormone synthesis. A DNA fragment encoding the mature form of human ferredoxin was cloned into an expression vector under control of the T7 **RNA polymerase**/promoter system. The protein was overproduced in Escherichia coli, and the [2Fe-2S] cluster was incorporated into the protein by in vitro reconstitution. The overall yield was approximately 30 mg of purified, reconstituted ferredoxin per liter of culture. Four of the five cysteines in human ferredoxin are coordinated to the iron-sulfur cluster. First, the non-ligand cysteine (cysteine-95) was mutated to alanine, and then double mutants were created in which each of the other four cysteines (at positions 46, 52, 55, and 92) were mutated individually to serine. The wild-type ferredoxin and each of the five mutant proteins were studied by UV-visible spectroscopy and electron paramagnetic resonance spectroscopy. The EPR gav values of all five mutants were very similar to that of wild-type human ferredoxin. In the **reduced** state, three of the cysteine-to-serine mutants exhibited axial EPR spectra similar to that of wild-type, but one of the double mutants (C52S/C95A) exhibited a rhombic EPR spectrum. The UV-visible spectroscopic properties of the wild-type and the C95A mutant ferredoxins were identical, but those of the other cysteine-to-serine mutant proteins of human ferredoxin were quite different

from those of the wild-type protein and each other. These results, along with those from cysteine-to-serine mutations in other ferredoxins, provide the basis for a more comprehensive theoretical and practical understanding of the features important to the ligation of [2Fe-2S] clusters, although they do not yet permit determination of which two cysteines ligate Fe(II) and which ligate Fe(III) in the **reduced** protein.

4/3,AB/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08926331 96278957 PMID: 8663034
Expression and spectroscopic characterization of the hydrogenosomal [2Fe-2S] ferredoxin from the protozoan Trichomonas vaginalis.
Vidakovic M S; Fraczkiewicz G; Germanas J P
Department of Chemistry, University of Houston, Houston, Texas 77204-5641, USA.
Journal of biological chemistry (UNITED STATES) Jun 21 1996, 271 (25) p14734-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The heterologous expression and spectroscopic characterization of the [2Fe-2S] ferredoxin from the sexually transmitted human parasite Trichomonas vaginalis is described. Using oligonucleotide primers based on the deduced DNA sequence, the gene encoding the ferredoxin was amplified by **polymerase** chain reaction and cloned into a T7 **RNA polymerase** expression vector. Expression of the gene in Escherichia coli host HMS174(DE3) resulted in the high level production of the protein with the correctly assembled iron-sulfur cluster. The absorption, circular dichroism, resonance Raman, and EPR spectra of the recombinant protein revealed many differences from those of other [2Fe-2S] ferredoxins. The redox potential of the protein (-347 mV versus normal hydrogen electrode) was also determined. Whereas the amino acid sequence of T. vaginalis ferredoxin showed greatest homology to the [2Fe-2S] ferredoxins found in bacteria and vertebrate mitochondria which function in cytochrome P450 oxidation pathways, the spectroscopic properties showed substantial dissimilarity. Differences in the biophysical properties and function of T. vaginalis ferredoxin are proposed to result from the characteristic amino acid sequence of the parasite protein near the cysteine residues that ligate the valence-localized Fe(III) site of the **reduced** cluster.

4/3,AB/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08857969 96206047 PMID: 8654929
The **RNA** -binding protein HF-I, known as a host factor for phage Qbeta **RNA** replication, is essential for rpoS translation in Escherichia coli.
Muffler A; Fischer D; Hengge-Aronis R
Department of Biology, University of Konstanz, Germany.
Genes & development (UNITED STATES) May 1 1996, 10 (9) p1143-51, ISSN 0890-9369 Journal Code: 8711660
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The rpoS-encoded sigma(S) subunit of **RNA polymerase** in Escherichia coli is a global regulatory factor involved in several stress responses. Mainly because of increased rpoS translation and stabilization of sigma(S), which in nonstressed cells is a highly unstable protein, the cellular sigma(S) content increases during entry into stationary phase and

in response to hyperosmolarity. Here, we identify the hfq-encoded **RNA** -binding protein HF-I, which has been known previously only as a host factor for the replication of phage Qbeta **RNA**, as an essential factor for rpoS translation. An hfq null mutant exhibits strongly **reduced** sigma(S) levels under all conditions tested and is deficient for growth phase-related and osmotic induction of sigma(S). Using a combination of gene fusion analysis and pulse-chase experiments, we demonstrate that the hfq mutant is specifically impaired in rpoS translation. We also present evidence that the H-NS protein, which has been shown to affect rpoS translation, acts in the same regulatory pathway as HF-I at a position upstream of HF-I or in conjunction with HF-I. In addition, we show that expression and heat induction of the heat shock sigma factor sigma(32) (encoded by rpoH) is not dependent on HF-I, although rpoH and rpoS are both subject to translational regulation probably mediated by changes in mRNA **secondary structure** . HF-I is the first factor known to be specifically involved in rpoS translation, and this role is the first cellular function to be identified for this abundant ribosome-associated **RNA**-binding protein in E. coli.

4/3,AB/53 (Item 53 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08851650 96204506 PMID: 8628226
Cloning and molecular analysis of the bifunctional dihydrofolate **reductase** -thymidylate synthase gene in the ciliated protozoan Paramecium tetraurelia.
Schlichtherle I M; Roos D S; Van Houten J L
Department of Biology, University of Vermont, Burlington 05405-0086, USA.
Molecular & general genetics : MGG (GERMANY) Apr 10 1996, 250 (6) p665-73, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have cloned the first bifunctional gene dihydrofolate **reductase** -thymidylate synthase (DHFR-TS) from a free-living, ciliated protozoan, Paramecium tetraurelia, and determined its macronuclear sequence using a modified ligation-mediated **polymerase** chain reaction (PCR) that can be of general use in cloning strategies, especially where cDNA libraries are limiting. While bifunctional enzyme sequences are known from parasitic protozoa, none had previously been found in free-living protozoa. The AT-rich (68%) coding region spanning 1386 bp appears to lack introns. DHFR-TS localizes to a approximately 500 kb macronuclear chromosome and is transcribed as an mRNA of approximately 1.66 kb, predicted to encode a 53 kDa protein of 462 residues. The N-terminal one-third of the protein is encoded by DHFR, which is joined by a short junctional peptide of approximately 12 amino acids to the highly conserved C-terminal TS domain. Among known DHFR-TS sequences, the P. tetraurelia gene is most similar to that from Toxoplasma gondii, based on primary sequence and parsimony analyses. The predicted **secondary** protein **structure** is similar to those of previously crystallized monofunctional sequences.

4/3,AB/54 (Item 54 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08797005 96134828 PMID: 8555166
Effect of human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein on HIV-1 reverse transcriptase activity in vitro.
Ji X; Klarmann G J; Preston B D
Department of Biochemistry, University of Utah, Salt Lake City 84112, USA.
Biochemistry (UNITED STATES) Jan 9 1996, 35 (1) p132-43, ISSN

0006-2960 Journal Code: 0370623
Contract/Grant No.: P30 ES05022; ES; NIEHS; R29 CA48174; CA; NCI; RO1 AI34834; AI; NIAID; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Conversion of human immunodeficiency virus type 1 (HIV-1) genomic **RNA** to viral DNA is a requisite step in the virus life cycle. This conversion is catalyzed by reverse transcriptase (RT) associated with a large nucleoprotein complex composed of several viral proteins including nucleocapsid (NC). To better characterize the biochemical mechanisms of viral DNA synthesis, we overexpressed and purified recombinant HIV-1 NC and studied its effect on the activity and processivity of HIV-1 RT during polymerization of HIV-1 template sequences in vitro. The effect of NC on steady-state RT activity was dependent on the order of addition of reaction components. Addition of NC prior to formation of RT-primer.template-dNTP ternary complexes inhibited primer extension and **reduced** total product yields by slowing steady-state RT turnover. In contrast, addition of NC to preformed ternary complexes resulted in efficient primer extension and increased RT processivity at specific DNA template sites. NC stimulated polymerization (2-4 times) through eight of 13 sites examined in the cRRE region of HIV-1 env and increased the rate of polymerization through the D3/CTS region of HIV-1 pol 10 times. The data suggest that NC affects RT processivity by facilitating polymerization through regions of template **secondary structure** . Thus, NC functions as a single-strand binding (SSB)-like accessory replication factor for RT in vitro and may be part of a multicomponent retroviral replication complex.

4/3,AB/55 (Item 55 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08712384 96072777 PMID: 7578153
Alanine-scanning mutagenesis of human transcript elongation factor TFIIS.
Cipres-Palacin G; Kane C M
Department of Molecular and Cell Biology, University of California, Berkeley 94720-3202, USA.
Biochemistry (UNITED STATES) Nov 21 1995, 34 (46) p15375-80, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM34963; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

TFIIS is a transcription elongation factor that binds to **RNA polymerase** II and allows it to transcribe through a variety of transcriptional blockages by inducing cleavage near the 3' end of the nascent transcript. Although this cleavage reaction plays a key role in the process of reactivation of transcription by TFIIS, the exact mechanism by which TFIIS promotes readthrough by **RNA polymerase** II is not completely understood. We therefore undertook a systematic mutagenesis of the C-terminal half of TFIIS (delta TFIIS) to evaluate the contribution of charged residues in this region to induce transcript cleavage and promote readthrough in vitro. Twenty-two delta TFIIS alanine-scanning mutants were constructed by substitution of alanine for each amino acid in clusters of charged residues in the C-terminal half of HeLa TFIIS. The ability to induce transcript cleavage and readthrough of these mutants was tested in vitro using **RNA polymerase** II ternary elongation complexes arrested at a block to elongation. This alanine-scanning mutagenesis analysis allowed the identification of regions or residues important for the activity of TFIIS. Many of the mutants were **reduced** alike in both cleavage and readthrough activities. However, in several cases there was no simple correlation between these activity **reductions**.

4/3,AB/56 (Item 56 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08613434 95370292 PMID: 7543900
Reduced frameshift fidelity and processivity of HIV-1 reverse transcriptase mutants containing alanine substitutions in helix H of the thumb subdomain.
Bebenek K; Beard W A; Casas-Finet J R; Kim H R; Darden T A; Wilson S H; Kunkel T A
Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.
Journal of biological chemistry (UNITED STATES) Aug 18 1995, 270 (33) p19516-23, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have analyzed two human immunodeficiency virus (HIV-1) reverse transcriptase mutants of helix H in the thumb subdomain suggested by x-ray crystallography to interact with the primer strand of the template-primer. These enzymes, G262A and W266A, were previously shown to have greatly elevated dissociation rate constants for template-primer and to be much less sensitive to inhibition by 3'-azidodeoxythymidine 5'-triphosphate. Here we describe their processivity and error specificity. The results reveal that: (i) both enzymes have **reduced** processivity and lower fidelity for template-primer slippage errors, (ii) they differ from each other in sequence-dependent termination of processive synthesis and in error specificity, and (iii) the magnitude of the mutator effect relative to wild-type enzyme for deletions in homopolymeric sequences decreases as the length of the run increases. Thus amino acid substitutions in a subdomain thought to interact with the duplex template-primer confer a strand slippage mutator phenotype to a replicative DNA **polymerase**. This suggests that interactions between specific amino acids and the primer stem at positions well removed from the active site are critical determinants of processivity and fidelity. These effects, obtained in aqueous solution during catalytic cycling, are consistent with and support the existing crystallographic structural model.

4/3,AB/57 (Item 57 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08516949 95275819 PMID: 7756255
Identification of nucleotide binding sites in the poliovirus **RNA polymerase**.
Richards O C; Hanson J L; Schultz S; Ehrenfeld E
Department of Molecular Biology and Biochemistry, University of California, Irvine 92717, USA.
Biochemistry (UNITED STATES) May 16 1995, 34 (19) p6288-95, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: AI 17386; AI; NIAID; CA-62203; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Poliovirus **RNA polymerase** (3Dpol) was cross-linked to [32P]ribonucleoside triphosphates (NTPs) by **reduction** of oxidized NTP-protein complexes. Cross-linked complexes were digested with cyanogen bromide, and resulting peptides were fractionated by reverse-phase HPLC. 32P-Labeled peptides were purified by **secondary** HPLC fractionation and/or additional digestion with endoproteinases Glu-C, TPCK-trypsin, or Asp-N followed by another HPLC fractionation. N-Terminal sequences of the

major [32P]-peptides were determined, and approximate sizes of these peptides were obtained by SDS-polyacrylamide gel electrophoresis. Two major NTP binding sites in 3Dpol were found. One site was between Asp-266 and Met-286; possible binding residues in this fragment were Lys-276, Lys-278, or Lys-283. A second binding site was between Ala-57 and Met-74 with Lys-61 or Lys-66 as possible binding residues. Alignment of these regions on the known **structure** of HIV-1 reverse transcriptase allowed us to predict the position of the downstream nucleotide binding site in the conserved "fingers" subdomain present near the active site cleft of both **RNA** and DNA polymerases. The N-terminal nucleotide binding site is not contained within a region that is conserved among other polymerases.

4/3,AB/58 (Item 58 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08503059 95258314 PMID: 7739888
Ciliate telomerase **RNA** structural features.
McCormick-Graham M; Romero D P
Department of Pharmacology, School of Medicine, University of Minnesota, Minneapolis 55455, USA.
Nucleic acids research (ENGLAND) Apr 11 1995, 23 (7) p1091-7, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM 50861; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Telomerase **RNA** is an integral part of telomerase, the ribonucleoprotein enzyme that catalyzes the synthesis of telomeric DNA. The **RNA** moiety contains a templating domain that directs the synthesis of a species-specific telomeric repeat and may also be important for enzyme **structure** and/or catalysis. Phylogenetic comparisons of telomerase **RNA** sequences from various Tetrahymena spp. and hypotrich ciliates have revealed two conserved **secondary structure** models that share many features. We have cloned and sequenced the telomerase **RNA** genes from an additional six Tetrahymena spp. (T. vorax, T. borealis, T. australis, T. silvana, T. capricornis and T. paravorax). Inclusion of these sequences, most notably that from T. paravorax, in a phylogenetic comparative analysis allowed us to more narrowly define structural elements that may be necessary for a minimal telomerase **RNA**. A primary sequence element, positioned 5' of the template and conserved between all previously known ciliate telomerase RNAs, has been **reduced** from 5'-(C)UGUCA-3' to the 4 nt sequence 5'-GUCA-3'. Conserved **secondary** structural features and the impact they have on the general organization of ciliate telomerase RNAs is discussed.

4/3,AB/59 (Item 59 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08450049 95199357 PMID: 7534421
Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides.
Shirasaka T; Kavlick M F; Ueno T; Gao W Y; Kojima E; Alcaide M L; Chokekijchai S; Roy B M; Arnold E; Yarchoan R; et al
Experimental Retrovirology Section, National Cancer Institute, Bethesda, MD 20892.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 14 1995, 92 (6) p2398-402, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: AI27690; AI; NIAID; AI36144; AI; NIAID
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A set of mutations [Ala-62-->Val(A62V), V75I, F77L, F116Y, and Q151M] in the **polymerase** domain of reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) confers on the virus a **reduced** sensitivity to multiple antiretroviral dideoxynucleosides and has been seen in HIV-1 variants isolated from patients receiving combination chemotherapy with 3'-azido-3'-deoxythymidine (AZT) plus 2',3'-dideoxycytidine (ddC) or 2',3'-dideoxyinosine (ddI). The IC50 values of AZT, ddC, ddI, 2',3'-dideoxyguanosine, and 2',3'-didehydro-3'-deoxythymidine against an infectious clone constructed to include the five mutations were significantly higher than those of a wild-type infectious clone. The K1 value for AZT 5'-triphosphate determined for the virus-associated RT from a posttherapy strain was 35-fold higher than that of RT from a pretherapy strain. Detailed analysis of HIV-1 strains isolated at various times during therapy showed that the Q151M mutation developed first in vivo, at the time when the viremia level suddenly increased, followed by the F116Y and F77L mutations. All five mutations ultimately developed, and the viremia level rose even further. Analyses based on the three-dimensional **structure** of HIV-1 RT suggest that the positions where at least several of the five mutations occur are located in close proximity to the proposed dNTP-binding site of RT and the first nucleotide position of the single-stranded template.

4/3,AB/60 (Item 60 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08449077 95197669 PMID: 7534310

Self-coded 3'-extension of run-off transcripts produces aberrant products during in vitro transcription with T7 **RNA polymerase**.

Triana-Alonso F J; Dabrowski M; Wadzack J; Nierhaus K H
Max-Planck-Institut fur Molekulare Genetik, A.G. Ribosomen, Berlin, Federal Republic of Germany.

Journal of biological chemistry (UNITED STATES) Mar 17 1995, 270 (11) p6298-307, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

More than 70% of the **RNA** synthesized by T7 **RNA polymerase** during run-off transcription in vitro can be incorrect products, up to twice as long as the expected transcripts. Transcriptions with model templates indicate that false transcription is mainly observed when the correct product cannot form stable **secondary** structures at the 3'-end. Therefore, the following hypothesis is tested: after leaving the DNA template, the **polymerase** can bind a transcript to the template site and the 3'-end of the transcript to the product site and extend it, if the 3'-end is not part of a stable **secondary structure**. Indeed, incubation of purified transcripts with the **polymerase** in transcription conditions triggers a 3'-end prolongation of the **RNA** . When two RNAs of different lengths are added to the transcription mix, both generate distinct and specific patterns of prolonged **RNA** products without any interference, demonstrating the self-coding nature of the prolongation process. Furthermore, sequencing of the high molecular weight transcripts demonstrates that their 5'-ends are precisely defined in sequence, whereas the 3'-ends contain size-variable extensions which show complementarity to the correct transcript. Surprisingly, a **reduction** of the UTP concentration to 0.2-1.0 mM in the presence of 3.5-4.0 mM of the other NTPs leads to faithful transcription and good yields, irrespective of the nucleotide composition of the template.

4/3,AB/61 (Item 61 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08265940 95023903 PMID: 7937764
Flavin **reductase** : sequence of cDNA from bovine liver and tissue distribution.
Quandt K S; Hultquist D E
Department of Biological Chemistry, University of Michigan, Ann Arbor 48109-0606.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Sep 27 1994, 91 (20) p9322-6, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: AG-07046; AG; NIA; M01 RR00042; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Flavin **reductase** catalyzes electron transfer from **reduced** pyridine nucleotides to methylene blue or riboflavin, and this catalysis is the basis of the therapeutic use of methylene blue or riboflavin in the treatment of methemoglobinemia. A cDNA for a mammalian flavin **reductase** has been isolated and sequenced. Degenerate oligonucleotides, with sequences based on amino acid sequences of peptides derived from bovine erythrocyte flavin **reductase** , were used as primers in PCR to selectively amplify a partial cDNA that encodes the bovine **reductase**. The template used in the PCR was first strand cDNA synthesized from bovine liver total **RNA** using oligo(dT) primers. A PCR product was used as a specific probe to screen a bovine liver cDNA library. The sequence determined from two overlapping clones contains an open reading frame of 621 nucleotides and encodes 206 amino acids. The amino acid sequence deduced from the bovine liver flavin **reductase** cDNA matches the amino acid sequences determined for erythrocyte **reductase** -derived peptides, and the predicted molecular mass of 22,001 Da for the liver **reductase** agrees well with the molecular mass of 21,994 Da determined for the erythrocyte **reductase** by electrospray mass spectrometry. The amino acid sequence at the N terminus of the **reductase** has homology to sequences of pyridine nucleotide-dependent enzymes, and the predicted **secondary structure**, beta alpha beta, resembles the common nucleotide-binding structural motif. **RNA** blot analysis indicates a single 1-kilobase **reductase** transcript in human heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle.

4/3,AB/62 (Item 62 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08259249 95016516 PMID: 7931150
Cruciform **structure** of a DNA motif of parvovirus minute virus of mice (prototype strain) involved in the attenuation of gene expression.
Perros M; Spegelaere P; Dupont F; Vanacker J M; Rommelaere J
Unite d'Oncologie Moleculaire, CNRS URA1160, Institut Pasteur de Lille, France.
Journal of general virology (ENGLAND) Oct 1994, 75 (Pt 10) p2645-53, ISSN 0022-1317 Journal Code: 0077340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It has previously been reported that the region between nucleotides 259 and 383 immediately downstream from the P4 early promoter of parvovirus minute virus of mice, prototype strain (MVMp) is responsible for transcriptional attenuation. Attenuation results from the premature pausing of **RNA polymerase** II within this sequence (designated to as

att) and seems to depend on potential **RNA secondary structure** . To assess the attenuation capacity of att under near physiological conditions, the early transcription unit of MVMp was replaced by the chloramphenicol acetyltransferase reporter gene under control of the early P4 promoter, in the presence or absence of att. The resulting recombinant vectors were encapsidated in parvovirus particles and replicated in cells after co-infection with the wild-type virus. The att fragment **reduced** the rate of expression of the reporter gene by approximately threefold, confirming previously reported data from transfection experiments performed in the same cellular system. This attenuation factor is unexpectedly high, considering that the 'readthrough' fold of the nascent viral transcript is thermodynamically more stable than the 'attenuation' configuration. In an attempt to elucidate this point, we sought for the presence of **secondary** structures in the template DNA molecule. In vitro nuclease probing of viral dsDNA revealed that the att fragment had a cruciform configuration with both complementary strands folding into the computer-predicted stem-loop 'attenuation' **structure** . These observations lead us to propose that the **secondary structure** of the DNA template may prompt the formation of the 'attenuation' stem-loop in nascent mRNAs by bringing corresponding self-complementary sequences into close proximity.

4/3,AB/63 (Item 63 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08225767 94359807 PMID: 8078770
The processing of wild type and mutant forms of rat nuclear pre-tRNA(Lys) by the homologous RNase P.
Paisley T E; Van Tuyle G C
Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond 23298.
Nucleic acids research (ENGLAND) Aug 25 1994, 22 (16) p3347-53, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM 38297; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The 5' processing of rat pre-tRNA(Lys) and a series of mutant derivatives by rat cytosolic RNase P was examined. In standard, non-kinetic assays, mutant precursors synthesized in vitro with 5' leader sequences of 10, 17, 24, 25, and 46 nucleotides were processed to approximately equal levels and yielded precisely cleaved 5' processed intermediates with the normal 7-base pair aminoacyl stems. The construct containing the tRNA(Lys) with the 46-nucleotide leader was modified by PCR to give a series of pre-tRNA(Lys) mutants designed to measure the effect on processing by (1) substituting the nucleotide at the +1 position, (2) pairing and unpairing the +1 and +72 bases, (3) elongating the aminoacyl stem, and (4) disrupting the helix of the aminoacyl stem. Comparative kinetic analyses revealed that changing the wild type +1G to A, C, or U was well tolerated by the RNase P provided that compensatory changes at +72 created a base pair or a G.U noncanonical pair. Mutants with elongated aminoacyl stems that were produced either by inserting an additional base pair at +3:a + 69:a or by pairing the -1A with a +73U, were processed to yield 7-base pair aminoacyl stems, but with different efficiencies. The efficiency seen with the double insertion mutant was higher than even the wild type precursor, but the -1A-U + 73 mutant was a relatively poor substrate. Disrupting the aminoacyl stem helix by introducing a +7G G + 66 mispairing or by inserting a single G at the +3:a position dramatically **reduced** the processing efficiency, although the position of cleavage occurred precisely at the wild type cleavage site. However, the single insertion of a C at the +69:a position resulted in an efficiently cleaved precursor, but permitted a minor, **secondary** cleavage within the leader between the -6 and -5

nucleotides in addition to the dominant wild type scission.

4/3,AB/64 (Item 64 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08204480 94339835 PMID: 8061605
A beta-turn in alpha-amanitin is the most important structural feature for binding to **RNA polymerase** II and three monoclonal antibodies.
Baumann K; Zanotti G; Faulstich H
Max-Planck-Institut fur medizinische Forschung, Heidelberg, Germany.
Protein science : a publication of the Protein Society (UNITED STATES) May 1994, 3 (5) p750-6, ISSN 0961-8368 Journal Code: 9211750
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Four amatoxin-binding proteins with KD values in the nanomolar range, 3 monoclonal antibodies and **RNA polymerase** II, were studied with respect to their affinities to 24 alpha-amanitin derivatives with modified side chains. From KD values we estimated the amounts of binding energy that single side chains of the amatoxins contribute to complex formation. Ile6, previously identified by X-ray analysis to be part of a beta-turn (Kostansek EC, Lipscomb WN, Yocum RR, Thiessen WE, 1978, Biochemistry 17:3790-3795) proved to be of outstanding importance in all complexes. Replacement of the isoleucine with alanine **reduced** the affinity to all binding proteins to < 1%, suggesting a strong hydrophobic interaction. A strong effect was also seen when Gly5 was replaced with alanine, suggesting that the absence of a side chain in proximity to the beta-turn is likewise important. In addition to the beta-turn, each of the proteins showed at least 2 other points of strong contact formed by hydrogen bonds. Donors are the indole NH of 6'-hydroxy-Trp4 and OH of hydroxy-Pro2 and dihydroxy-Ile3. All the antibodies, but not **RNA polymerase** II, recognized the indole nucleus of 6'-hydroxy-Trp4. The geometric arrangement of the 4 strongest contact points suggests that the amatoxin binding site is different in each of the 4 proteins, except for the 2 antibodies raised in the same animal. Here, most of the contact points were identical but differed in strength of interaction. The method of structural analysis presented in this study is useful for identifying contact sites in complexes of proteins with peptides of rigid conformation. Furthermore, the method complements X-ray data by providing information on the amount of binding energy contributed by single structural elements.

4/3,AB/65 (Item 65 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08200305 94334977 PMID: 8057359
A molecular rheostat. Co-operative rev binding to stem I of the rev-response element modulates human immunodeficiency virus type-1 late gene expression.
Mann D A; Mikaelian I; Zemmel R W; Green S M; Lowe A D; Kimura T; Singh M ; Butler P J; Gait M J; Karn J
MRC Laboratory of Molecular Biology, Cambridge, U.K.
Journal of molecular biology (ENGLAND) Aug 12 1994, 241 (2) p193-207 , ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The complete biologically active human immunodeficiency virus type-1 (HIV-1) rev-response element (RRE) **RNA** is 351 nucleotides (nt) in length, and includes an extra 58 nt on the 5' end and 59 nt on the 3' end

beyond the sites proposed in the original models for the RRE **secondary structure** . The extra sequences are able to form a duplex **structure** which extends Stem I. The presence of an elongated Stem I **structure** in the RRE **RNA** was confirmed by nuclease mapping experiments. Nuclease protection experiments have shown that rev binds to restricted regions of the RRE, including the high affinity site located at the base of Stem IIb and along the length of the Stem I region. The three large stem-loop structures which protrude from Stem I and Stem IIb (Stems IIc, III+IV and V) remain accessible to nucleases even in the presence of a large excess of protein. Gel-retardation experiments show that the truncations of Stem I **reduced** the total number of rev molecules that can bind co-operatively and with high affinity to the RRE **RNA**. To test whether the elongated Stem I **structure** is required for maximal rev activity, a series of truncations which progressively **reduced** the length of Stem I was introduced into an HIV-1 derived reporter plasmid. In the presence of rev and a functional RRE, there is an increase in the levels of gag and env mRNA in the cytoplasm and a decrease in levels of tat and rev mRNAs. Each of the truncations in Stem I **reduced** the rev responses, with the longest truncations producing the greatest losses of activity. The data suggest that the RRE acts as a "molecular rheostat" designed to detect rev levels during the early stages of the HIV growth cycle.

4/3,AB/66 (Item 66 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08116265 94237867 PMID: 7514172
Requirements of the **secondary** structures in the primary transcript for multicopy single-stranded DNA synthesis by reverse transcriptase from bacterial retron-Ec107.
Shimada M; Inouye S; Inouye M
Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854.
Journal of biological chemistry (UNITED STATES) May 20 1994, 269 (20) p14553-8, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM44012; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Multicopy single-stranded DNA (msDNA) is produced by bacterial retroelements called retrons. It consists of single-stranded DNA that is linked to an internal G residue of an **RNA** molecule by a 2',5'-phosphodiester linkage. It has been demonstrated that specific primary sequences, as well as the **secondary** structures immediately downstream of the G residue, are essential for the cDNA priming reaction (Shimamoto, T., Hsu, M.-Y., Inouye, S., and Inouye, M. (1993) J. Biol. Chem. 268, 2684-2692). We have now examined the requirement of the structures in the region corresponding to DNA for msDNA synthesis. The upper stem region consisting of 71 bases of msDNA-Ec107 was found not to be essential, and this region could be deleted to efficiently produce a truncated msDNA containing only a 36-base single-stranded DNA. Various mutations including base replacements, deletions, and insertions were constructed in the lower stem region. It was found that any mutations resulting in more stable **secondary** structures caused **reduction** in msDNA synthesis. The results indicated that reverse transcriptase requires a loose **secondary structure** in the template **RNA** near the cDNA priming site for cDNA elongation.

4/3,AB/67 (Item 67 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08085850 94233778 PMID: 8178491
The proximate 5' and 3' ends of the 120-base viral **RNA** (pRNA) are crucial for the packaging of bacteriophage phi 29 DNA.
Zhang C; Lee C S; Guo P
Department of Veterinary Pathobiology, Purdue University, West Lafayette, Indiana 47907.
Virology (UNITED STATES) May 15 1994, 201 (1) p77-85, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: GM46490; GM; NIGMS; GM48159; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In vitro mutagenesis was performed to identify the DNA packaging domain of the 120-base pRNA essential and specific for DNA encapsidation by bacteriophage phi 29 of Bacillus subtilis. All deletions and mutations targeted the 5' and 3' ends of the pRNA. DNA templates of a control or mutant pRNAs used for in vitro transcription with T7 **RNA polymerase** were generated by PCR. Fourteen mutant pRNA molecules were synthesized from DNA templates either directly after PCR or after cloning the PCR fragments into the pCR II vector. Ten of the mutant pRNA species were inactive in packaging of the phi 29 genome. Mutation of base one at the 5' end did not affect the pRNA packaging activity. Mutation of the first two bases at the 5' end of the pRNA to noncomplementary bases in the predicted **RNA secondary structure** (U1 C2/A117G116 to G1 G2/A117G116) resulted in a pRNA with no detectable DNA-gp3 packaging activity assayed by either sucrose gradient sedimentation or agarose gel electrophoresis, and 10(5)-fold **reduction** in activity was found when measured by plaque-forming units with a new highly sensitive assay system. Changing bases 116 and 117 so that they were complementary to the mutated bases, 1 and 2, from the previous mutant (G1 G2/A117G116 to G1 G2/C117C116) generated an **RNA** molecule with restored DNA packaging ability. Our results show that, although not essential for procapsid binding, both the 5' and 3' ends of the pRNA were proximate and crucial for phi 29 DNA packaging.

4/3,AB/68 (Item 68 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08080360 94214451 PMID: 8162033
Complete androgen insensitivity due to mutations in the probable alpha-helical segments of the DNA-binding domain in the human androgen receptor.
Beitel L K; Prior L; Vasiliou D M; Gottlieb B; Kaufman M; Lumbroso R; Alvarado C; McGillivray B; Trifiro M; Pinsky L
Lady Davis Institute, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, Canada.
Human molecular genetics (ENGLAND) Jan 1994, 3 (1) p21-7, ISSN 0964-6906 Journal Code: 9208958
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We describe different single-amino acid aberrations in the DNA-binding domain (DBD) of the human androgen receptor (hAR) in three families with complete androgen insensitivity. No additional alteration was found in the translated portion of each mutant gene. In one family, an in-frame 3 nt deletion removes codon 581-(or 582) and, thereby, one of two phenylalanines that invariably occupy adjacent positions in the N-terminal alpha-helical region of the DBD in the steroid/thyroid/vitamin D receptor superfamily. In the second, an in-frame 3 nt loss deletes Arg614, an invariant residue in the C-terminal alpha-helix of the DBD. In the third, a G-->A transition causes Arg614His. Following transient transfection of COS cells with each

mutant AR plasmid, there is a normal concentration of specific androgen-binding activity that has a **reduced** ability to bind two types of androgen response element (ARE), and to transregulate an androgen-responsive human growth hormone reporter gene. In genital skin fibroblasts with delta Phe581 or Arg614His, androgen-binding, AR protein and AR mRNA are markedly **reduced**; in gonadal fibroblasts with delta Arg614, AR mRNA may be **reduced**. Our data substantiate the primary contributions of Phe581 and Arg614 to normal hAR-ARE binding, and expose important **secondary** effects of the mutations affecting each residue.

4/3,AB/69 (Item 69 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08053081 94180390 PMID: 8133519
Bacteriophage T7 **RNA polymerase** and its active-site mutants. Kinetic, spectroscopic and calorimetric characterization.
Osumi-Davis P A; Sreerama N; Volkin D B; Middaugh C R; Woody R W; Woody A Y
Department of Biochemistry, Colorado State University, Fort Collins 80523.
Journal of molecular biology (ENGLAND) Mar 18 1994, 237 (1) p5-19, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM-23697; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It has been demonstrated that the amino acids Asp537, Asp812, Lys631, His811 and Tyr639 are involved in bacteriophage T7 **RNA polymerase** catalysis. In the present paper, we report kinetic, spectroscopic and calorimetric characterization of the wild-type and mutant T7 **RNA** polymerases generated at these five loci (D537N, E; K631M, R; Y639F, S, A, W; H811Q, A; D812N, E). The wild-type enzyme has a substantial amount of **secondary structure** as determined by CD analysis (alpha-helix, 43%; beta-sheet, 14%; beta-turn, 25%; unordered, 18%). The CD spectra of 12 mutants at five loci are very similar to that of the wild-type, except for the mutant Y639W. Within experimental error, the thermal transition temperatures measured by CD and DSC as well as the lambda max values of the fluorescence spectra were the same for the wild-type and all of the mutants. Therefore, the overall folding and stability of the mutant enzymes are very similar to those of the wild-type enzyme, although small local conformational changes cannot be excluded. For the synthesis of the pentamer pppGGACU, the mutants D537E and D812E showed an approximately two- to threefold decrease in (kcat)app and an approximately two- to threefold increase in (Km)app, relative to the wild-type, in contrast to the mutants D537N and D812N which exhibited no detectable activity. The mutant K631R showed a sevenfold **reduction** in (kcat)app and a two- to threefold increase in (Km)app, supporting our earlier observation with the mutant K631M that Lys631 may be involved in phosphodiester bond formation. The mutant Y639S can synthesize the trimer GGA with an approximately 50-fold decrease in (kcat)app and a tenfold increase in (Km)app, relative to the wild-type, underlining the importance of the phenyl ring of Tyr639. The mutant H811A, in which the side-chain at position 811 is incapable of forming a hydrogen bond, can synthesize the trimer GGA with an approximately tenfold decrease in (kcat)app and an approximately 35-fold increase in (Km)app. Thus, either the hydrogen-bonding capacity of this residue is non-essential or some other group can functionally substitute for the His811 side-chain. The wild-type enzyme showed significant effects of the base position in the sequence on the apparent binding constants for the NTPs. The kinetics of GpG-primed trimer, tetramer and pentamer synthesis on three 22 bp templates were investigated for the wild-type and mutant enzymes with measurable activity. (ABSTRACT TRUNCATED AT 400 WORDS)

4/3,AB/70 (Item 70 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07971522 94111993 PMID: 8284218
An unstructured mRNA region and a 5' hairpin represent important elements of the E. coli translation initiation signal determined by using the bacteriophage T7 gene 1 translation start site.
Helke A; Geisen R M; Vollmer M; Sprengart M L; Fuchs E
University of Heidelberg, Germany.
Nucleic acids research (ENGLAND) Dec 11 1993, 21 (24) p5705-11, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Gene 1 of bacteriophage T7 early region--the **RNA polymerase** gene--is very actively translated during the infectious cycle of this phage. A 29 base pair fragment of its ribosome binding site containing the initiation triplet, the Shine-Dalgarno sequence (S-D), 10 nucleotides (nt) upstream and 6 nt downstream of these central elements was cloned into a vector to control the expression of the mouse dihydrofolate **reductase** gene (dhfr). Although all essential parts of this translation initiation region (TIR) should be present, this fragment showed only very low activity. Computer analysis revealed a potentially inhibitory hairpin binding the S-D sequence into its stem base paired to vector-derived upstream sequences. Mutational alterations demonstrated that this hairpin was not responsible for the low activity. However, addition of 21 nt of the T7 gene 1 upstream sequence to the 29 base pair fragment were capable of increasing the translational efficiency by one order of magnitude. Computer analysis of this sequence, including nucleotide shuffling, revealed that it contains a highly unstructured region lacking mRNA **secondary** structures but with a hairpin at its 5' end, here formed solely by T7 sequences. There was not much difference in activity whether the mRNA included or lacked vector-derived sequences upstream of the hairpin. Such highly unstructured mRNA regions were found in all very efficiently expressed T7 genes without any obvious sequence homologies. The delta G values of these regions were higher, i.e. potential **secondary** structural elements were fewer, than in TIR of genes from E. coli. This is likely due to the fact that T7 as a lytic phage is relying for successful infection on much stronger signals which a cell cannot afford because of the indispensable balanced equilibria of its interdependent biochemical processes. When the 5' ends of efficient T7 gene mRNA are formed by the action of RNase III they generally start with an unstructured region. Efficiently expressed T7 genes within a polycistronic mRNA, however, always contain a hairpin preceding the **structure** free sequence. We suggest that the formation of this 5' hairpin is releasing enough energy to keep the unstructured regions free of **secondary RNA** structures for sufficient time to give ribosomes and factors a good chance for binding to the TIR. In addition, sequences further downstream of the start codon give rise to an additional increase in efficiency of the TIR by almost two orders of magnitude.

4/3,AB/71 (Item 71 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07943210 94080020 PMID: 8257796
The Caenorhabditis elegans homologue of the extracellular calcium binding protein SPARC/osteonectin affects nematode body morphology and mobility.
Schwarzbauer J E; Spencer C S
Department of Molecular Biology, Princeton University, NJ 08544-1014.
Molecular biology of the cell (UNITED STATES) Sep 1993, 4 (9)

p941-52, ISSN 1059-1524 Journal Code: 9201390
Contract/Grant No.: CA-44627; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The extracellular matrix-associated protein, SPARC (osteonectin [Secreted Protein Acidic and Rich in Cysteine]), modulates cell adhesion and induces a change in cell morphology. SPARC expression in mammals is developmentally regulated and is highest at sites of extracellular matrix assembly and remodeling such as parietal endoderm and bone. We have isolated cDNA and genomic DNA clones encoding the Caenorhabditis elegans homologue of SPARC. The gene organization is highly conserved, and the proteins encoded by mouse, human, and nematode genes are about 38% identical. SPARC consists of four domains (I-IV) based on predicted **secondary structure**.
Using bacterial fusion proteins containing nematode domain I or the domain IV EF-hand motif, we show that, like the mammalian proteins, both domains bind calcium. In transgenic nematodes expressing a SPARC-lacZ fusion gene, beta-galactosidase staining accumulated in a striated pattern in the more heavily stained muscle cells along the body. Comparison of the pattern of transgene expression to unc-54-lacZ animals demonstrated that SPARC is expressed by body wall and sex muscle cells. Appropriate levels of SPARC are essential for normal C. elegans development and muscle function. Transgenic nematodes overexpressing the wild-type SPARC gene were abnormal. Embryos were deformed, and adult hermaphrodites had vulval protrusions and an uncoordinated (Unc) phenotype with **reduced** mobility and paralysis.

4/3,AB/72 (Item 72 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07727765 93252935 PMID: 8486701
A cationic region of the platelet-derived growth factor (PDGF) A-chain (Arg159-Lys160-Lys161) is required for receptor binding and mitogenic activity of the PDGF-AA homodimer.
Fenstermaker R A; Poptic E; Bonfield T L; Knauss T C; Corsillo L; Piskurich J F; Kaetzel C S; Jentoft J E; Gelfand C; DiCorleto P E; et al
Department of Veterans Affairs Medical Center, Cleveland, Ohio 44106.
Journal of biological chemistry (UNITED STATES) May 15 1993, 268 (14) p10482-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Platelet-derived growth factor-AA and -BB homodimers and -AB heterodimers bind with high affinity to the platelet-derived growth factor (PDGF) alpha-receptor. Basic polypeptides such as polylysine and protamine sulfate compete with PDGF for receptor binding, suggesting a role for ligand positive charge in the binding interaction. A pentapeptide amino acid sequence with a cationic tripeptide core is perfectly conserved between the A- and B-chains (Val158-Arg159-Lys160-Lys161-Pro162) and was therefore considered as a possible alpha-receptor-binding domain. We have investigated the functional importance of positive charge within this region of the PDGF A-chain by using site-directed mutagenesis to convert the cationic core amino acids to the acidic sequence triglutamic acid. cDNAs encoding wild-type (PDGF-AAwt) and charge mutant (PDGF-AAcm) proteins were expressed following stable transfection of Chinese hamster ovary cells. Proper assembly and secretion of PDGF-AAcm was verified by metabolic labeling with [35S]cysteine, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis analysis under nonreducing and **reducing** conditions. PDGF-AAcm was secreted as two major species of disulfide-linked A-chain homodimers identical in molecular mass to those observed for PDGF-AAwt (32 and 35 kDa). Secreted PDGF-AAwt and PDGF-AAcm proteins were purified to homogeneity and subjected to structural and functional

analyses. Compared to purified PDGF-AAwt, PDGF-AAcm displayed a marked **reduction** in both binding affinity for PDGF alpha-receptors and mitogenic activity in Swiss 3T3 cells. Large **reductions** were also observed in the ability of semipurified PDGF-AAcm to stimulate calcium influx and the production of inositol phosphates. Measurement of circular dichroism spectra of highly purified PDGF-AAcm and PDGF-AAwt revealed no significant difference in **secondary structure**. Collectively, these results indicate that the cationic Arg159-Lys160-Lys161 region plays a critical role in the biological activity of PDGF-AA by direct participation in ligand binding to the PDGF alpha-receptor.

4/3,AB/73 (Item 73 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07727688 93252858 PMID: 7683663
Template-directed pausing of DNA synthesis by HIV-1 reverse transcriptase during polymerization of HIV-1 sequences in vitro.
Klarmann G J; Schauber C A; Preston B D
Laboratory for Cancer Research, College of Pharmacy, Rutgers University, Piscataway, New Jersey 08854.
Journal of biological chemistry (UNITED STATES) May 5 1993, 268 (13) p9793-802, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: P30 ES05022; ES; NIEHS; R29 CA48174; CA; NCI; S15 CA51339; CA; NCI
Erratum in J Biol Chem 1993 Jun 25;268(18) 13764
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Replication of human immunodeficiency virus type 1 (HIV-1) requires reverse transcriptase (RT) to synthesize double-stranded proviral DNA (9.7 kilobases) through a complex mechanism utilizing both **RNA** and DNA templates. We have examined DNA synthesis by HIV-1 RT on **RNA** and DNA templates derived from the HIV-1 genome using a primer extension assay in vitro. Analysis of polymerization products on sequencing gels revealed strong pauses in synthesis, on both **RNA** and DNA templates, in homopolymeric nucleotide runs, and at regions of predicted **secondary structure** . Polymerization pauses occurred in runs of template rGs (> or = 4 bases) and rCs (> or = 3 bases) during minus-strand synthesis on **RNA** templates, and in most runs (> or = 4 bases) of template dTs and dAs during plus-strand synthesis on DNA templates. Pausing also occurred on both templates within the first few nucleotides of the predicted hairpin structures of the Rev response element. The locations of pauses were dependent on template sequence and were unaffected by primer positioning, RT concentration, and ionic strength. Recombinant and virion-derived HIV-1 RTs showed similar pausing patterns. DNA products that accumulated at HIV-1 RT pause sites on **RNA** templates were extended by continued incubation with excess RT from Moloney murine leukemia virus, showing that the **RNA** templates were not broken or otherwise unable to support polymerization. Polymerizations conducted in the presence of a poly(rA) oligo(dT) trap showed that pausing results from two mechanisms: 1) RT remaining bound to the primer-template and polymerizing at a greatly **reduced** rate, or 2) RT dissociating from the primer-template. These results demonstrate that specific HIV-1 **RNA** and DNA template sequences are capable of interrupting processive DNA synthesis by HIV-1 RT in vitro. Pausing may serve specific functions in HIV-1 replication and mutagenesis. Moreover, these data suggest that one or more accessory factors are required to complete proviral DNA synthesis in vivo and that efficient HIV-1 DNA synthesis may require multiple origins.

4/3,AB/74 (Item 74 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07694554 93217987 PMID: 8464061
Structural studies of the enveloped dsRNA bacteriophage phi 6 of Pseudomonas syringae by Raman spectroscopy. II. Nucleocapsid **structure** and thermostability of the virion, nucleocapsid and **polymerase** complex.
Bamford J K; Bamford D H; Li T; Thomas G J
Department of Genetics, University of Helsinki, Finland.
Journal of molecular biology (ENGLAND) Mar 20 1993, 230 (2) p473-82, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: AI11855; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Structures and thermostabilities of the double-stranded (ds) **RNA** bacteriophage phi 6 and of its isolated nucleocapsid-**polymerase** complex (nucleocapsid core) and dsRNA components have been investigated by Raman spectroscopy. The spectra show that proteins of the phi 6 virion are collectively deficient in beta-sheet **secondary structure**. In particular, the major protein (P8) of the outer spherical shell of the phi 6 nucleocapsid exhibits a **secondary structure** dominated largely by alpha-helix and irregular conformations. The absence of appreciable beta-**structure** in the P8 subunit suggests a tertiary conformation lacking the beta-barrel motif common to subunits of most other spherical viral capsids. In addition, the Raman spectra show that subunits of the dodecahedral nucleocapsid core are also predominantly alpha-helical. The results thus indicate a largely alpha-helical **secondary structure** for the major subunit (P1) of the phi 6 nucleocapsid core, as well as for the P8 subunit of the outer spherical shell. Using Raman difference spectroscopy, we demonstrate that proteins of the nucleocapsid core (P1, P2, P4 and P7) interact extensively with the packaged phi 6 **RNA** genome, and further, that conformational stability of the packaged **RNA** is **reduced** upon removal from the core. Also, we find that proteins of the phi 6 nucleocapsid are significantly more thermostable than proteins of the viral membrane envelope, which are reported in the accompanying paper (Li et al., 1993). The present results suggest that both the architectural principles and modes of protein-**RNA** interaction in the phi 6 virion differ fundamentally from those of icosahedral single-stranded **RNA** viruses. Both Raman and circular dichroism spectra indicate that the dsRNA genome of phi 6 is an A-form **structure** . The Raman marker bands signify the presence only of C3'-endo/anti nucleoside conformers. The Raman signature of dsRNA, revealed in the spectrum of the phi 6 genome, is discussed here as a model for assessing base-pairing and base-stacking interactions in other ribonucleoprotein assemblies.

4/3,AB/75 (Item 75 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07618502 93143360 PMID: 8380971
Purification and characterization of recombinant-expressed cytochrome P450 2C3 from Escherichia coli: 2C3 encodes the 6 beta-hydroxylase deficient form of P450 3b.
Richardson T H; Hsu M H; Kronbach T; Barnes H J; Chan G; Waterman M R; Kemper B; Johnson E F
Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, California 92037.
Archives of biochemistry and biophysics (UNITED STATES) Jan 1993, 300 (1) p510-6, ISSN 0003-9861 Journal Code: 0372430
Contract/Grant No.: GM31001; GM; NIGMS; GM35897; GM; NIGMS; GM37942; GM; NIGMS; +
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Rabbit cytochrome P450 2C3 was expressed from its cDNA in Escherichia coli as a chimeric enzyme in which a portion of the N-terminal membrane anchor sequence of 2C3 was replaced with a modified sequence derived from P450 17 alpha. The nucleotide sequence encoding the N-terminus of P450 17 alpha was modified previously to achieve a high level of expression of P450 17 alpha in E. coli by altering the first eight codons of P450 17 alpha to reflect second codon preferences for high expression and to minimize the potential for the formation of a stable **secondary structure** of the corresponding **RNA** transcript. The modified P450 2C3 was expressed at > 400 nmol/liter of culture. P450 2C3 was isolated to apparent electrophoretic homogeneity and a specific content > 14 nmol P450/mg protein. When reconstituted with P450 **reductase** and dilauroyl-L-alpha-lecithin, the purified E. coli-expressed P450 2C3 catalyzed 16 alpha, but not 6 beta-hydroxylation of progesterone. Expression of unmodified 2C3 from its cDNA in COS-1 cells confirmed the absence of detectable 6 beta-hydroxylase activity. In addition, the enzyme expressed in E. coli is activated by the allosteric effector 5 beta-pregnane-3 beta,20 alpha-diol, with a resultant Vmax = 10 min-1 and Km = 20 microM and is not inhibited by 16 alpha-methylprogesterone. These results indicate that the 2C3 cDNA encodes an enzymatic form characteristic of IIIvo/J and B/J inbred rabbits rather than a second enzymatic form expressed in most outbred and some inbred strains that catalyzes both high efficiency 16 alpha- and 6 beta-hydroxylation of progesterone. Our results have identified the enzyme variant encoded by the 2C3 cDNA and have demonstrated the utility of E. coli for the expression of recombinant P450 enzymes.

4/3,AB/76 (Item 76 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07611906 93123161 PMID: 8419292

Suppression of ColE1 high-copy-number mutants by mutations in the polA gene of Escherichia coli.

Yang Y L; Polisky B
Department of Biology, Indiana University, Bloomington 47405.

Journal of bacteriology (UNITED STATES) Jan 1993, 175 (2) p428-37, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: GM24212; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We isolated three Escherichia coli suppressor strains that **reduce** the copy number of a mutant ColE1 high-copy-number plasmid. These mutations lower the copy number of the mutant plasmid in vivo up to 15-fold; the wild-type plasmid copy number is **reduced** by two- to threefold. The suppressor strains do not affect the copy numbers of non-ColE1-type plasmids tested, suggesting that their effects are specific for ColE1-type plasmids. Two of the suppressor strains show ColE1 allele-specific suppression; i.e., certain plasmid copy number mutations are suppressed more efficiently than others, suggesting specificity in the interaction between the suppressor gene product and plasmid replication component(s). All of the mutations were genetically mapped to the chromosomal polA gene, which encodes DNA **polymerase** I. The suppressor mutational changes were identified by DNA sequencing and found to alter single nucleotides in the region encoding the Klenow fragment of DNA **polymerase** I. Two mutations map in the DNA-binding cleft of the **polymerase** region and are suggested to affect specific interactions of the enzyme with the replication primer **RNA** encoded by the plasmid. The third suppressor alters a residue in the 3'-5' exonuclease domain of the enzyme.

Implications for the interaction of DNA **polymerase** I with the ColE1 primer **RNA** are discussed.

4/3,AB/77 (Item 77 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07587359 93100796 PMID: 7677953
Proteolytic processing of Ty3 proteins is required for transposition.
Kirchner J; Sandmeyer S
Department of Microbiology and Molecular Genetics, University of California, Irvine 92717-4025.
Journal of virology (UNITED STATES) Jan 1993, 67 (1) p19-28, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: GM07311; GM; NIGMS; GM33281; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Ty3 is a retroviruslike element found in Saccharomyces cerevisiae. It encodes GAG3 and GAG3-POL3 polyproteins which are processed into mature proteins found in the Ty3 viruslike particle. In this study, the region encoding a protease that is homologous to retroviral aspartyl proteases was identified and shown to be required for production of mature Ty3 proteins and transposition. The Ty3 protease has the Asp-Ser-Gly consensus sequence found in copia, Ty1, and Rous sarcoma virus proteases, rather than the Asp-Thr-Gly found in most retroviral proteases. The Asp-Ser-Gly consensus is flanked by residues similar to those which flank the active sites of cellular aspartyl proteases. Mutations were made in the Ty3 active-site sequence to examine the role of the protease in Ty3 particle maturation and to test the functional significance of the Ser active-site variant in the consensus sequence. Mutation of the active-site Asp blocked processing of Gag3 and Gag3-Pol3 and allowed identification of a GAG3-POL3 polyprotein. This protein was turned over rapidly in cells expressing the mutant Ty3. Changing the active-site Ser to Thr caused only a modest **reduction** in the levels of certain Ty3 proteins. Five putative cleavage sites of this protease in Ty3 GAG3 and GAG3-POL3 polyproteins were defined by amino-terminal sequence analysis. The existence of an additional protein(s) of unknown function, encoded downstream of the protease-coding region, was deduced from the positions of these amino termini and the sizes of known Ty3 proteins. Although Ty3 protease cleavage sites do not correspond exactly to known retroviral protease cleavage sites, there are similarities. Residues P3 through P2' in the regions encompassing each of the five sites are uncharged, and no P1 position is occupied by an amino acid with a branched beta carbon.

4/3,AB/78 (Item 78 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07581795 93110352 PMID: 1470913
Selection of a ribozyme that functions as a superior template in a self-copying reaction.
Green R; Szostak J W
Department of Molecular Biology, Massachusetts General Hospital, Boston 02114.
Science (UNITED STATES) Dec 18 1992, 258 (5090) p1910-5, ISSN 0036-8075 Journal Code: 0404511
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The sunY ribozyme is derived from a self-splicing **RNA** group I intron. This ribozyme was chosen as a starting point for the design of a

self-replicating **RNA** because of its small size. As a means of facilitating the self-replication process, the size of this ribozyme was decreased by the deletion of nonconserved structural domains; however, when such deletions were made, there were severe losses of enzymatic activity. In vitro genetic selection was used to identify mutations that reactivate a virtually inactive sunY deletion mutant. A selected mutant with five substitution mutations scattered throughout the primary sequence showed greater catalytic activity than the original ribozyme under the selection conditions. The sunY ribozyme and its small selected variant can both catalyze template-directed oligonucleotide assembly. The small size and **reduced secondary structure** of the selected variant results in an enhancement, relative to that of the original ribozyme, of its rate of self-copying. This engineered ribozyme is able to function effectively both as a catalyst and as a template in self-copying reactions.

4/3,AB/79 (Item 79 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07483633 93010939 PMID: 1396552
Primary and **secondary structure** of the pore-forming peptide of pathogenic Entamoeba histolytica.
Leippe M; Tannich E; Nickel R; van der Goot G; Pattus F; Horstmann R D; Muller-Eberhard H J
Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.
EMBO journal (ENGLAND) Oct 1992, 11 (10) p3501-6, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A pore-forming peptide is implicated in the potent cytolytic activity of pathogenic Entamoeba histolytica. Using NH2-terminal sequence information of this peptide, the corresponding cDNA was isolated. The cDNA-deduced amino acid sequence revealed a putative signal peptide and a mature peptide of 77 amino acids including six cysteine residues. Computer-aided **secondary structure** analysis predicted that the peptide would be composed of four adjacent alpha-helices, and CD spectroscopy indicated an all alpha-helical conformation. The tertiary **structure** appears to be stabilized by three disulfide bonds; the pore-forming activity was not sensitive to heat but was lost in the presence of **reducing** agents. Sequence homology was found to the saposins and to surfactant-associated protein B, both mammalian polypeptides of similar size and **secondary structure** but of non-lytic function. In particular, the six cysteine residues were found to be conserved, suggesting a common motif for stabilizing a favourable tertiary **structure**. Compared with previously characterized toxic peptides also containing three disulfide bonds, the amoeba peptide may represent a distinct class of biologically active peptides.

4/3,AB/80 (Item 80 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07275090 92202197 PMID: 1313012
Glu327 is part of a catalytic triad in rat liver fructose-2,6-bisphosphatase.
Lin K; Li L; Correia J J; Pilkis S J
Department of Physiology and Biophysics, State University of New York, Stony Brook 11794-8661.
Journal of biological chemistry (UNITED STATES) Apr 5 1992, 267 (10) p6556-62, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: DK38354; DK; NIDDK; GM41117; GM; NIGMS

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The fructose-2,6-bisphosphatase domain of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase has been shown to be structurally and functionally homologous to phosphoglycerate mutase. Both enzymes catalyze their reactions via phosphoenzyme intermediates which utilize an active site histidine as a nucleophilic phosphoacceptor and another histidine as a proton donor to the leaving group. Glu327 in the bisphosphatase domain of the rat liver bifunctional enzyme is conserved in all phosphoglycerate mutase structures and is postulated, by modelling studies, to be located in the active site. Glu327 was mutated to Ala, Gln, or Asp. The mutant and wild-type enzymes were expressed in Escherichia coli with a T-7 **RNA polymerase** -based expression system and purified to homogeneity by substrate elution from phosphocellulose. The Glu327 mutants had apparent molecular weights of 110,000 by gel filtration and had unaltered 6-phosphofructo-2-kinase activity. Circular dichroism showed that the **secondary structure** of the Glu327 mutant enzyme forms was the same as the wild-type enzyme. The maximal velocity of the fructose-2,6-bisphosphatase of the Glu327----Ala, Glu327----Gln, and Glu327----Asp mutants was 4, 2, and 20%, respectively, that of the wild-type enzyme, but the rate of phosphoenzyme formation of the mutants was **reduced** by at least a factor of 1000. In addition, the rate constants of phosphoenzyme hydrolysis for the Glu372----Ala and Glu327----Gln mutants were 2.7 and 1.3%, respectively, of the wild type, whereas the rate constant for the Glu327----Asp mutant was 60% of the wild-type value. Glu327 was not a substrate or product binding site determinant since the Km for fructose-2,6-bisphosphate and Ki for fructose-6-phosphate of the mutants were not appreciably changed. The results implicate Glu327 as part of a catalytic triad in fructose-2,6-bisphosphatase and suggest that it influences the protonation state of the active site histidine residues during phosphoenzyme formation and/or acts as a base catalyst to enhance the nucleophilic attack of water on the phosphoenzyme intermediate.

4/3,AB/81 (Item 81 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07275086 92202193 PMID: 1313010

Lysine 274 is essential for fructose 2,6-bisphosphate inhibition of fructose-1,6-bisphosphatase.

el-Maghrabi M R; Austin L R; Correia J J; Pilkis S J
Department of Physiology and Biophysics, State University of New York, Stony Brook 11794.

Journal of biological chemistry (UNITED STATES) Apr 5 1992, 267 (10) p6526-30, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: DK 38354; DK; NIDDK; GM 41117; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Lysine 274 is conserved in all known fructose-1,6-bisphosphatase sequences. It has been implicated in substrate binding and/or catalysis on the basis of reactivity with pyridoxal phosphate as well as by x-ray crystallographic analysis. Lys274 of rat liver fructose-1,6-bisphosphatase was mutated to alanine by the **polymerase** chain reaction, and the T7-**RNA polymerase** -transcribed construct containing the mutant sequence was expressed in Escherichia coli. The mutant and wild-type forms of the enzyme were purified to homogeneity, and their specific activity, substrate dependence, and inhibition by fructose 2,6-bisphosphate and AMP were compared. While the mutant exhibited no change in maximal velocity, its Km for fructose 1,6-bisphosphate was 20-fold higher than that of the

wild-type, and its Ki for fructose 2,6-bisphosphate was increased 1000-fold. Consistent with the unaltered maximal velocity, there were no apparent difference between the **secondary structure** of the wild-type and mutant enzyme forms, as measured by circular dichroism and ultraviolet difference spectroscopy. The Ki for the allosteric inhibitor AMP was only slightly increased, indicating that Lys274 is not directly involved in AMP inhibition. Fructose 2,6-bisphosphate potentiated AMP inhibition of both forms, but 500-fold higher concentrations of fructose 2,6-bisphosphate were needed to **reduce** the Ki for AMP for the mutant compared to the wild-type. However, potentiation of AMP inhibition of the Lys274----Ala mutant was evident at fructose 2,6-bisphosphate concentrations (approximately 100 microM) well below those that inhibited the enzyme, which suggests that fructose 2,6-bisphosphate interacts either with the AMP site directly or with other residues involved in the active site-AMP synergy. The results also demonstrate that although Lys274 is an important binding site determinant for sugar bisphosphates, it plays a more significant role in binding fructose 2,6-bisphosphate than fructose 1,6-bisphosphate, probably because it binds the 2-phospho group of the former while other residues bind the 1-phospho group of the substrate. It is concluded that the enzyme utilizes Lys274 to discriminate between its substrate and fructose 2,6-bisphosphate.

4/3,AB/82 (Item 82 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07208406 92144737 PMID: 1782268
[Biochemical mechanisms of resistance to a new antineoplastic drug CRC 680578 from the nitrosourea class]
Biokhimicheskie mekhanizmy rezistentnosti k novomu protivoopukholevomu preparatu iz klassa nitrozomochevin CRC 680578.
Gudtsova K V; Kukushkina G V; Gorbacheva L B
Biokhimii a (Moscow, Russia) (USSR) Aug 1991, 56 (8) p1509-21, ISSN 0320-9725 Journal Code: 0372667
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
The biochemical mechanisms of resistance to CRC 680578, a new antitumour chloroethylnitrosourea alpha-amino acid derivative, were studied. Alterations in DNA, **RNA** and protein syntheses, SH-group content, drug efflux, activities of replicative and repair enzymes, such as ribonucleotide **reductase**, thymidine kinase, O6-alkylguanine-DNA-alkyl transferase and DNA polymerases alpha and beta and damages of the DNA **secondary structure** were investigated in sensitive and resistant to CRC 680578 leukemia L1210 cells. It was found that the total SH-group number in drug-resistant cells was increased (about 1.3-fold in comparison with sensitive cells) which seems to be due to the mechanisms of drug resistance. CHC 680578 induced less pronounced inhibition and more rapid restoration of DNA and **RNA** synthesis in resistant cells. No differences between the ribonucleotide **reductase** and thymidine kinase activities were found either in intact cells of the both strains or after drug administration. The efficiency of repair of DNA chloroethyl adducts by O6-alkylguanine-DNA-alkyltransferase in leukemia cells of various sensitivity was found to be identical. The differences in enzyme activities in intact cells of the both strains were insignificant. It was supposed that factors other than changes in the level of O6-alkylguanine-DNA-alkyltransferase in leukemia cells may be responsible for the resistance to CRC 680578. The increase in the levels of DNA **polymerase** alpha and, especially, of DNA **polymerase** beta, in sensitive (but not resistant) mouse leukemia cells 48 hours after drug administration is though to define the mechanism of resistance to the new antitumour agent CHC 680578.

4/3,AB/83 (Item 83 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07084255 92015208 PMID: 1920404
Ribosomal **RNA** identity elements for ricin A-chain recognition and catalysis.
Endo Y; Gluck A; Wool I G
Department of Biochemistry, Yamanashi Medical College, Japan.
Journal of molecular biology (ENGLAND) Sep 5 1991, 221 (1) p193-207, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM33702; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Ricin is a cytotoxic protein that inactivates ribosomes by hydrolyzing the N-glycosidic bond between the base and the ribose at position A4324 in eukaryotic 28 S rRNA. The requirements for the recognition by ricin A-chain of this nucleotide and for the catalysis of cleavage were examined using a synthetic oligoribonucleotide that reproduces the sequence and the **secondary structure** of the **RNA** domain (a helical stem, a bulged nucleotide, and a 17-member single-stranded loop). The wild-type **RNA** (35mer) and a number of mutants were transcribed in vitro from synthetic DNA templates with phage T7 **RNA polymerase**. With the wild-type oligoribonucleotide the ricin A-chain catalyzed reaction has a Km of 13.55 microM and a Kcat of 0.023 min-1. Recognition and catalysis by ricin A-chain has an absolute requirement for A at the position that corresponds to 4324. The helical stem is also essential; however, the number of base-pairs can be **reduced** from the seven found in 28 S rRNA to three without loss of identity. The nature of these base-pairs can affect catalysis. A change of the second set from one canonical (G.C) to another (U.A) **reduces** sensitivity to ricin A-chain; whereas, a change of the third pair (U.A----G.C) produces supersensitivity. The bulged nucleotide does not contribute to identification. Hydrolysis is affected by altering the nucleotides in the universal sequence surrounding A4324 or by changing the position in the loop of the tetranucleotide GA(ricin)GA: all of these mutants have a null phenotype. If ribosomes are treated first with alpha-sarcin to cleave the phosphodiester bond at G4325 ricin can still catalyze depurination at A4324. This implies that cleavage by alpha-sarcin at the center of what has been presumed to be a 17 nucleotide single-stranded loop in 28 S rRNA produces ends that are constrained in some way. On the other hand, hydrolysis by alpha-sarcin of the corresponding position in the synthetic oligoribonucleotide prevents recognition by ricin A-chain. The results suggest that the loop has a complex **structure**, affected by ribosomal proteins, and this bears on the function in protein synthesis of the alpha-sarcin/ricin rRNA domain.

4/3,AB/84 (Item 84 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06942641 91251240 PMID: 1710292
Overlapping retrovirus U5 sequence elements are required for efficient integration and initiation of reverse transcription.
Cobrinik D; Aiyar A; Ge Z; Katzman M; Huang H; Leis J
Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.
Journal of virology (UNITED STATES) Jul 1991, 65 (7) p3864-72, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA 38046; CA; NCI; P30 CA 43703; CA; NCI; T32 GM 07250; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed

A **secondary structure** in the 5' noncoding region of avian retrovirus **RNA**, called the U5-leader stem, was shown previously to have a role in initiation of reverse transcription (D. Cobrinik, L. Soskey, and J. Leis, J. Virol. 62:3622-3630, 1988). We now show that an additional **RNA secondary structure** near the U5 terminus, called the U5-IR stem, is also important for reverse transcription. Mutations that disrupt the U5-IR stem cause a replication defect associated with both a decrease in synthesis of viral DNA in infected cells and a decrease in initiation of reverse transcription in melittin-permeabilized virions. **Structure**-compensating base substitutions in the U5-IR restore reverse transcription efficiency. In viral DNA, U5-IR sequences are included in the U5 terminal region that functions as a viral integration donor site. When base substitutions are introduced into these sequences, a **reduced** efficiency of integration in vitro and in vivo is observed. These observations indicate that U5-IR sequences have a structural role in reverse transcription of viral **RNA** and a sequence-specific role in the integration of viral DNA.

4/3,AB/85 (Item 85 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06803342 91105080 PMID: 1988020

Preparation and characterization of N-(1-pyrenyl)iodoacetamide-labeled Escherichia coli **RNA polymerase**.

Johnson R S; Bowers M; Eaton Q
Department of Biochemistry, East Carolina University School of Medicine, Greenville, North Carolina 27858.

Biochemistry (UNITED STATES) Jan 8 1991, 30 (1) p189-98, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

N-(1-Pyrenyl)iodoacetamide has been used to introduce fluorescent probes into Escherichia coli **RNA polymerase**. After an incubation time of 15 min, approximately 2 pyrene equiv was introduced per enzyme molecule. There was no further increase in modification after more extended periods of incubation. Neither calf thymus DNA nor nucleotides protected the holoenzyme from modification. Thus, the sites of modification do not appear to involve the binding sites for polynucleotides or the ribonucleoside triphosphates. From the isolation and analysis of the individual subunits, it was found that sigma contained approximately 1 pyrene equiv, beta contained 0.6, beta' contained 0.6, and alpha less than 0.1. Spectral and Stern-Volmer analyses indicate that the covalently attached pyrene molecules are in comparable apolar microenvironments. On the basis of CD analyses, the introduction of pyrene molecules into **RNA polymerase** alters its **secondary structure**. This alteration in **secondary structure** manifests itself by a **reduction** in overall enzymatic activity. Transcript analysis of the products obtained by using a linearized plasmid containing the A1 promoter and the Te terminator of bacteriophage T7 indicates that the pyrenyl derivative is capable of producing full-length transcripts and that it has an efficiency of chain termination comparable to the native enzyme. Analysis of tau plots for the interaction of the pyrenyl derivative and the native enzyme, respectively, with the A1 promoter yielded comparable values for the isomerization constant in the conversion of the closed complex to an open one. Comparable values were also obtained for the association constant. The rate of chain elongation for the pyrenyl derivative, however, is approximately 54% of that observed for the native enzyme. Thus, the decrease in overall transcriptional activity observed with the pyrenyl derivative is not due to a decrease in the efficiency of initiation or

premature termination, but rather to a decrease in the rate of chain elongation.

4/3,AB/86 (Item 86 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06752331 91062382 PMID: 2247461
A spliced leader is present on a subset of mRNAs from the human parasite Schistosoma mansoni.
Rajkovic A; Davis R E; Simonsen J N; Rottman F M
Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, OH 44106.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 1990, 87 (22) p8879-83, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: 5T32GM07250; GM; NIGMS; AI15351; AI; NIAID; DK32770; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We present evidence that a subset of mRNAs in the human parasitic trematode Schistosoma mansoni contain an identical 36-nucleotide spliced leader (SL) sequence at their 5' termini. The SL is derived from a 90-nucleotide nonpolyadenylylated **RNA** (SL **RNA**), presumably by trans-splicing. Neither the SL nor the SL **RNA** share significant sequence identity with previously described trans-spliced leaders and SL RNAs in trypanosomatid protozoans or nematodes. However, several features, such as predicted **secondary structure**, trimethylguanosine cap, and potential Sm binding site, suggest similarities among SL RNAs in widely divergent organisms. Our evidence also indicates that the exon 3 acceptor site of the 3-hydroxy-3-methylglutaryl-CoA **reductase** gene can be spliced either to the SL by trans-splicing or to an upstream exon, 2, by cis-splicing. The presence of a SL sequence in S. mansoni, a member of the phylum Platyhelminthes, suggests that transplicing may be a common feature of other lower invertebrates.

4/3,AB/87 (Item 87 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06743538 91056566 PMID: 2243382
Human immunodeficiency virus rev protein recognizes a target sequence in rev-responsive element **RNA** within the context of **RNA** **secondary structure**.
Holland S M; Ahmad N; Maitra R K; Wingfield P; Venkatesan S
Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892.
Journal of virology (UNITED STATES) Dec 1990, 64 (12) p5966-75, ISSN 0022-538X Journal Code: 0113724
Erratum in J Virol 1992 Feb;66(2) 1288
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Human immunodeficiency virus type 1 Rev protein modulates the distribution of viral mRNAs from the nucleus to the cytoplasm by interaction with a highly structured viral **RNA** sequence, the Rev-responsive element (RRE). To identify the minimal functional elements of RRE, we evaluated mutant RREs for Rev binding in vitro and Rev response in vivo in the context of a Gag expression plasmid. The critical functional elements fold into a **structure** composed of a stem-loop A, formed by

the ends of the RRE, joined to a branched stem-loop B/B1/B2, between bases 49 and 113. The 5' 132 nucleotides of RRE, RREDDE, which possessed a similar **structure** , bound Rev efficiently but were nonfunctional in vivo, implying separate binding and functional domains within the RRE. Excision of stem-loop A **reduced** Rev binding significantly and abolished the in vivo Rev response. The B2 branch could be removed without severe impairment of binding, but deletions in the B1 branch significantly **reduced** binding and function. However, deletion of 12 nucleotides, including the 5' strand of stem B, abolished both binding and function, while excision of the 3' strand of stem B only **reduced** them. Maintenance of the native RRE **secondary structure** alone was not sufficient for Rev recognition. Many mutations that altered the primary **structure** of the critical region while preserving the original **RNA** conformation were Rev responsive. However, mutations that changed a 5'..CACUAUGGG..3' sequence in the B stem, without affecting the overall **structure** abolished both in vitro Rev binding and the in vivo Rev response.

4/3,AB/88 (Item 88 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06427724 90130478 PMID: 2298746
RNA-protein interaction. An analysis with **RNA** oligonucleotides of the recognition by alpha-sarcin of a ribosomal domain critical for function.
Endo Y; Gluck A; Chan Y L; Tsurugi K; Wool I G
Department of Biochemistry, Yamanashi Medical College, Japan.
Journal of biological chemistry (UNITED STATES) Feb 5 1990, 265 (4) p2216-22, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM-33702; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
alpha-Sarcin is a cytotoxic protein that inactivates ribosomes by hydrolyzing a single phosphodiester bond on the 3' side of G-4325 in eukaryotic 28 S rRNA. We have examined the requirements for the recognition by alpha-sarcin of this domain using a synthetic oligoribonucleotide (35-mer) that reproduces the sequence and, we presume, the **secondary structure** (a stem, a bulged nucleotide, and a loop) at the site of modification. The wild type **structure** and a large number of variants were transcribed in vitro from synthetic DNA templates with phage T7 **RNA polymerase** . Recognition of the substrate is strongly favored by a G at the position that corresponds to 4325. There is an absolute requirement for a helical stem; however, it can be **reduced** from the 7 base pairs in the natural **structure** to 3 without loss of specificity. The nature of the base pairs in the stem modifies but does not abolish recognition; whereas, the bulged nucleotide does not contribute to identification. Cleavage is materially affected by altering the nucleotides in the universal sequence surrounding G-4325 and changing the position in the loop of the tetranucleotide GAG(sarcin)A leads to loss of recognition by the toxin. We propose that the alpha-sarcin domain **RNA** participates in elongation factor catalyzed binding of aminoacyl-tRNA and of translocation; that translocation is driven by transitions in the **structure** of the alpha-sarcin domain **RNA** initiated by the binding of the factors, or the hydrolysis of GTP, or both; and that to toxin inactivates the ribosomes by preventing this transition.

4/3,AB/89 (Item 89 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06269527 89359253 PMID: 2670923

Attenuation in the regulation of the pyrBI operon in Escherichia coli. In vivo studies of transcriptional termination.
Levin H L; Park K; Schachman H K
Department of Molecular Biology, University of California, Berkeley 94720.
Journal of biological chemistry (UNITED STATES) Sep 5 1989, 264 (25) p14638-45, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM 12159; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The attenuation model for transcriptional regulation of the Escherichia coli pyrBI operon is based on the assumption that transcription terminates upstream of the structural genes at a rho-independent terminator when cells contain high levels of UTP. When, however, the cells are limited for pyrimidines, the presence of ribosomes translating the short leader peptide is presumed to cause an alteration in the **secondary structure** of the terminator in a way that allows **RNA polymerase** to transcribe the entire operon. These two premises of transcriptional regulation were tested by using exonuclease protection assays to map the 3' ends of transcripts extracted from cells containing either ample or depleted concentrations of pyrimidines. The results support the model since 99% of the pyrBI transcripts terminated at the (G + C)-rich region of dyad symmetry upstream of the structural genes when cells were grown in excess uracil. In addition, a significant portion (36%) of the pyrBI transcripts extracted from cells containing **reduced** pyrimidine concentrations extended past the dyad into the structural genes. This observation correlated with the amounts of aspartate transcarbamoylase synthesized in cells under the various conditions. The mapping technique was also used to determine the position of the 5' ends of the transcripts to measure contributions of two potential start sites (P1 and P2) to the pool of pyrBI transcripts. The results show that under all conditions no more than 3% of the total transcripts had 5' ends corresponding to the upstream promoter, P1. In cells lacking P1 virtually all transcripts from P2 terminated at the (G + C)-rich hairpin when the cellular level of pyrimidines was high. Conversely 57% of the transcripts extended past the terminator when cells were grown in UMP. The S1 nuclease technique also provided a measure of the steady state level of transcripts originating at P2. In cells depleted of pyrimidines there was a 5-10-fold increase in these transcripts depending on the number of copies of pyrBI. This increase, which is independent of attenuation, is caused by a different regulatory mechanism which as yet has not been identified.

4/3,AB/90 (Item 90 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06259129 89345160 PMID: 2762152
Mutagenesis analysis of a self-cleaving **RNA**.
Sheldon C C; Symons R H
Department of Biochemistry, University of Adelaide, Australia.
Nucleic acids research (ENGLAND) Jul 25 1989, 17 (14) p5679-85, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The hammerhead structural model proposed for sequences that mediate self-cleavage of certain RNAs contains base-paired three stems and 13 conserved bases. Insertion, deletion and base substitution mutations were carried out on a 58 base **RNA** containing the sequence of the single-hammerhead **structure** of the plus **RNA** of the virusoid of lucerne transient streak virus, and the effects on self-cleavage assessed.

Results showed that there is flexibility in the sequence requirements for self-cleavage in vitro, but alterations of the conserved sequence or predicted **secondary structure** generally **reduced** the efficiency of self-cleavage.

4/3,AB/91 (Item 91 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06259118 89345149 PMID: 2668890
Transcription regulation in vitro by an E. coli promoter containing a DNA cruciform in the '-35' region.
Horwitz M S
Department of Pathology, School of Medicine, University of Washington, Seattle 98195.
Nucleic acids research (ENGLAND) Jul 25 1989, 17 (14) p5537-45, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM07266; GM; NIGMS; R35CA39903; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A promoter with the potential to adopt a 50 basepair (bp) cruciform spanning from -19 to -69 has been constructed in the plasmid pBR322 tetracycline resistance gene (tet) by forming an inverted repeat from '-35' sequences. Compared to a control promoter, the sequence of this cruciform promoter differs only by a 22 bp insertion between -48 and -69, upstream from the usual location of promoter sequences. The cruciform is extruded in a supercoil-dependent manner, and transcription from this promoter in vitro by **RNA polymerase** decreases as the negative supercoil density of the plasmid DNA increases. In contrast, transcription from the control promoter increases with negative supercoiling. Thus, DNA **secondary structure** in the '-35' region can affect promoter-**polymerase** interaction. The tet promoter cruciform also influences expression of the pBR322 beta-lactamase gene (bla). This apparently results when extrusion of the cruciform **reduces** the superhelicity of the plasmid molecule to a level that is below the optimum for expression from the bla promoter, illustrating one mechanism for how DNA **secondary structure** may effect action-at-a-distance. Transcription from both promoters in vivo does not differ from controls, suggesting that this cruciform is not generated to a significant extent intracellularly, most probably as a result of the slow kinetics of extrusion.

4/3,AB/92 (Item 92 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06124779 89224171 PMID: 3508341
Human **RNA polymerase** II can prematurely terminate transcription of the adenovirus type 2 late transcription unit at a precise site that resembles a prokaryotic termination signal.
Seiberg M; Kessler M; Levine A J; Aloni Y
Department of Genetics, Weizmann Institute of Science, Rehovot, Israel.
Virus genes (UNITED STATES) Nov 1987, 1 (1) p97-116, ISSN 0920-8569 Journal Code: 8803967
Contract/Grant No.: CA 14995; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Premature termination of transcription has been demonstrated by eukaryotic **RNA polymerase** II at specific sites in the major late transcriptional unit of SV40 and in one of the transcriptional units of the parvovirus, minute virus of mice (MVM) (Y. Aloni and N. Hay, CRC

Critical Reviews of Biochem., 18:327-383, 1985). In both cases the prematurely terminated (attenuated) **RNA** can be folded into a hairpin **structure** followed by U-residues that resemble a termination signal in prokaryotes. The experiments presented herein demonstrate premature termination of transcription 185 nucleotides (nt) downstream from the major late promoter of adenovirus type 2 (Ad2) in vivo, and in vitro in isolated nuclei and in HeLa whole cell extract. As in SV40 and MVM the attenuated **RNA** of Ad2 can be folded into a hairpin **structure** followed by U-residues. Transcription-termination was significantly **reduced** when ITP replaced GTP and when Br-UTP replaced UTP in the transcription reaction mixture, indicating that **RNA secondary structure** and the rU-dA interactions, respectively, are parts of the termination signal. Moreover, in isolated nuclei transcription-termination at the attenuation site occurred when the reaction mixture contained between 50-150 mM NaCl but not when it contained 300 mM NaCl. These results indicate that, at least in isolated nuclei, attenuation can be regulated. The possible involvement of termination factor(s) in the regulation of attenuation is discussed.

4/3,AB/93 (Item 93 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05875926 88296477 PMID: 2841127
Studies on the mechanism of translational enhancement by the 5'-leader sequence of tobacco mosaic virus **RNA**.
Sleat D E; Hull R; Turner P C; Wilson T M
Department of Virus Research, John Innes Institute and Agricultural and Food Research Council Institute of Plant Science Research, Norwich, England.
European journal of biochemistry / FEBS (GERMANY, WEST) Jul 15 1988, 175 (1) p75-86, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Translation of foreign mRNAs is enhanced by a cis-acting derivative (omega') of the 5'-leader sequence (omega) of tobacco mosaic virus **RNA** (vulgare strain). To explain this effect we have conducted several experiments in vitro. 1. The presence of various 5'-terminal sequences, including omega', did not significantly increase the half-lives of chloramphenicol acetyltransferase (CAT) or neomycin phosphotransferase (NPTII) mRNAs in wheat-germ extract. Also, a long leader sequence, unrelated to omega', did not enhance expression of NPTII mRNA in vitro. 2. The ability of several leader sequences, including omega', to form multiple initiation complexes with 80S (wheat germ) ribosomes was examined using CAT or NPTII mRNAs incubated in the presence of sparsomycin. Formation of disome complexes was unrelated to the capacity of a 5'-leader sequence to enhance translation. 3. Expression of CAT mRNA in both wheat germ extract and messenger-dependent rabbit reticulocyte lysate was less susceptible to inhibition by increasing salt concentration when a 5'-proximal omega' sequence was present. This effect was less marked when the CAT mRNA was capped. Conversely at high salt concentrations, capping was less stimulatory for mRNA with a 5'-proximal omega' sequence. These data suggest that omega' and the cap enhance translation, at least in part, by a similar mechanism. We propose that both features **reduce RNA secondary structure** , thereby rendering the 5' terminus more accessible to scanning by 40S ribosomal subunits and/or interaction with associated initiation factors. This conclusion was supported by computer-based **secondary-structure** analyses of our SP6 **RNA polymerase** transcript sequences. The ability of 5' leader sequences from brome mosaic virus **RNA** 3, alfalfa mosaic virus **RNA** 4, and the genomic RNAs of turnip yellow mosaic virus, Rous sarcoma virus or tobacco mosaic virus (tomato strain) to enhance mRNA

translation in eukaryotic systems may also be correlated with their respective **secondary** structures. A different mechanism probably accounts for the omega'-dependent enhancement of mRNA expression in Escherichia coli or in E. coli cell-free systems.

4/3,AB/94 (Item 94 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05669382 88087133 PMID: 2961747
Contributions of **RNA secondary structure** and length of the thymidine tract to transcription termination at the thr operon attenuator.
Lynn S P; Kasper L M; Gardner J F
Department of Microbiology, University of Illinois 61801.
Journal of biological chemistry (UNITED STATES) Jan 5 1988, 263 (1) p472-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The thr operon of Escherichia coli is regulated by an attenuation mechanism in which regulated transcription termination occurs in response to the levels of charged tRNAthr and tRNAile. Transcription of the thr operon regulatory region in vitro produces a 162-base transcript that is terminated efficiently at the attenuator. The attenuator sequence is similar to other rho-independent terminators. It contains a G + C region of dyad symmetry followed by a run of 9 A + T residues. We have characterized in detail the sequence requirements for efficient transcription termination in vitro. Using a set of point mutations in the G + C region of dyad symmetry of the thr attenuator, we have characterized the effects of these mutations on the efficiency of transcription termination. The efficiency was **reduced** in all of the mutants analyzed with the greatest effect being an approximate 20% decrease in termination. In some instances the electrophoretic mobilities of the terminated transcripts on 8% polyacrylamide, 8 M urea gels were shifted substantially relative to the wild type-terminated transcript, but the sites of transcription termination were altered by only a few base pairs. We also constructed a set of deletions removing consecutive thymidines which follow the G + C-rich region of dyad symmetry. Removal of 1 or 3 of the 9 thymidine residues had no effect on termination efficiency in vitro or in vivo. Removal of four to six thymidines caused a linear decrease in the efficiency of termination. When only one or two thymidines were present in the template, termination was completely abolished. These results indicate that both the integrity of the **RNA** stem and the length of the consecutive thymidine residues are important signals recognized by **RNA polymerase** during transcription of the thr operon regulatory region.

4/3,AB/95 (Item 95 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05588031 88011325 PMID: 2821285
Mapping and characterization of transcriptional pause sites in the early genetic region of bacteriophage T7.
Levin J R; Chamberlin M J
Department of Biochemistry, University of California, Berkely 94720.
Journal of molecular biology (ENGLAND) Jul 5 1987, 196 (1) p61-84, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM12010; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

During transcription of DNA templates in vitro, Escherichia coli **RNA polymerase** pauses at certain sequences before resuming elongation. Previous studies have established that some pausing events are brought about by the formation of **RNA** hairpin structures in the nascent transcript; however, it is not known whether this is an invariant and causal relationship. We have mapped and characterized almost 200 distinct pause sites located within the early region of bacteriophage T7 DNA using a collection of T7 deletion mutant DNAs and taking advantage of a procedure that permits synchronous transcription from the T7 A1 promoter. The pausing pattern is sensitive both to the overall concentration of nucleotide substrates and to the relative concentrations of the four nucleotides. The apparent Ks value for a particular nucleoside triphosphate can vary over a 500-fold range depending on the nucleotide sequence, and pausing at some sites can be induced by modest **reductions** in substrate concentrations. However, pausing is not solely a consequence of substrate limitation. Pausing at certain sites is caused by some feature of the template or of the transcript itself. Substitution of inosine triphosphate (ITP) for GTP during transcription strongly affects the pattern and strength of pausing events, suggesting that base-pairing interactions involving the **RNA** strand are important for some pausing events. Other pauses are determined by sequences downstream from the elongation site that have not yet been transcribed, and pausing at these sites is generally insensitive to substitution of IMP for GMP in the nascent transcript. Pausing at one particular site on T7 DNA is strongly enhanced by the presence of E. coli gene nusA protein. These results confirm that there are multiple classes of sites that lead to transcriptional pausing, and provide a collection of sites for further study. Using selected pause sites in the early region of T7 DNA, we have tried to evaluate the possible roles of primary sequence, base composition and **secondary structure** in pausing. Computer analysis was used to compare primary sequences and potential **RNA** hairpin structures in transcripts for pauses known to share similar biochemical properties. We see no correlation of pause sites with regions of particular base composition or with specific primary sequences. While some pauses are correlated with the potential to form stable **RNA** hairpins just upstream from the growing point of the **RNA** chain, there is not a strict one-to-one relationship between predicted **RNA** hairpins and the location of pause sites.(ABSTRACT TRUNCATED AT 400 WORDS)

4/3,AB/96 (Item 96 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04601296 84289613 PMID: 6206069
Stability of an **RNA secondary structure** affects in vitro transcription pausing in the trp operon leader region.
Landick R; Yanofsky C
Journal of biological chemistry (UNITED STATES) Sep 25 1984, 259 (18) p11550-5, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM 09738; GM; NIGMS; GM-09151-02; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Transcription of the tryptophan (trp) operon of Escherichia coli and other bacterial species is regulated by the formation of alternative **secondary** structures in the leader segment of the transcript. During in vitro transcription of the trp leader region **RNA polymerase** pauses at base pair 92 after synthesis of an **RNA** hairpin **secondary structure** . We studied the dependence of pausing on hairpin stability by examining mutant trp templates containing base pair substitutions in the region corresponding to the hairpin **secondary structure** . Base changes that lower the stability of the hairpin were found to **reduce** both the frequency and half-life of **RNA**

polymerase pausing while base changes that do not affect hairpin stability had little effect on pausing. Pausing was enhanced by the nusA protein; this enhancement was greatly **reduced** on mutant templates specifying less stable hairpins. The frequency of pausing on some mutant templates was correlated with the extent of read-through transcription beyond the trp attenuator, suggesting a possible role for pausing in the coupling of transcription and translation during transcription of the leader region of the operon.

4/3,AB/97 (Item 97 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04572880 84261462 PMID: 6378628
RNA secondary structure and translation inhibition: analysis of mutants in the rplJ leader.
Christensen T; Johnsen M; Fiil N P; Friesen J D
EMBO journal (ENGLAND) Jul 1984, 3 (7) p1609-12, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have carried out measurements of the stable binding of the ribosomal protein (r-protein) complex L10-L7/L12 to mutant forms of the mRNA leader of the rplJ operon of Escherichia coli. One of the point mutations, base 1548, which lies within the L10-L7/L12-protected region, almost completely abolishes in vitro formation of a stable complex of L10-L7/L12 with rplJ mRNA leader, and a second point mutation, base 1634, strongly **reduces** it. These observations constitute strong support for the proposition that L10-L7/L12 binds to the rplJ leader in bringing about translational feedback. To account for the action of these and other mutations, and to explain the mechanism of translation feedback inhibition, we suggest a **secondary structure** model involving alternate forms of the rplJ mRNA leader.

4/3,AB/98 (Item 98 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04455803 84144038 PMID: 6322106
Attenuation in SV40 as a mechanism of transcription-termination by **RNA polymerase** B.
Hay N; Aloni Y
Nucleic acids research (ENGLAND) Feb 10 1984, 12 (3) p1401-14, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: CA 14995; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Nuclei which were isolated from SV40 infected cells with a hypotonic detergent-free buffer were used to establish in vitro conditions which lead to transcription-termination at the attenuation site of SV40. This system allowed us to identify regulatory elements involved in transcription-termination by **RNA polymerase** B transcribing SV40. Transcription-termination at the attenuation site was found to be ionic strength dependent. Efficient termination occurred at low (100 mM NaCl) but not at high (100 mM (NH4)2 SO4 or 300 mM NaCl) ionic strength. When nuclei were prewashed with 300 mM NaCl, the efficiency of transcription-termination was low even when transcription was carried out at low ionic strength (100 mM NaCl). Efficient transcription-termination in the high salt prewashed nuclei was reconstituted by complementation with a high salt (300 mM NaCl) soluble factor extracted from nuclei of uninfected

cells. In addition, the efficiency of transcription-termination was significantly **reduced** when ITP replaced GTP in the transcription reaction mixture. Our data indicate that a nuclear factor and **RNA secondary structure** are essential regulatory elements involved in transcription-termination by **RNA polymerase** B.

4/3,AB/99 (Item 99 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03980227 82247965 PMID: 6179092
Transcription termination at the tryptophan operon attenuator is decreased in vitro by an oligomer complementary to a segment of the leader transcript.
Winkler M E; Mullis K; Barnett J; Stroynowski I; Yanofsky C
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1982, 79 (7) p2181-5, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A DNA oligomer 15 nucleotides long was used to probe the involvement of **RNA secondary structure** in the control of transcription termination at the attenuator of the tryptophan (trp) operon of Escherichia coli. This 15-mer is perfectly complementary to a segment of trp **RNA** that is thought to play a role in regulation of attenuation. When added to an in vitro transcription reaction mixture containing wild-type E. coli or Salmonella typhimurium trp operon templates, the complementary 15-mer caused a 4-fold increase in read-through transcription. By contrast, the 15-mer did not affect attenuation when a mutant E. coli template was used that does not allow formation of a crucial **RNA secondary structure** . Control experiments established that oligomers that were not complementary to E. coli trp leader **RNA** did not affect attenuation and that the 15-mer did not **reduce** termination when the transcript lacked a complementary region. Other experiments established that the 15-mer did not increase read-through transcription by allowing **RNA polymerase** molecules that might have already stopped at the attenuator to resume transcription. These findings provide direct support for the view that alternate base-paired structures control transcription termination at the trp attenuator.

4/3,AB/100 (Item 100 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02535582 77113900 PMID: 837369
Effect of N-2-acetylaminofluorene modification on the **structure** and template activity of DNA and reconstituted chromatin.
Yamasaki H; Leffler S; Weinstein I B
Cancer research (UNITED STATES) Mar 1977, 37 (3) p684-91, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This study compares the effects of in vitro modification of native duck reticulocyte DNA by [14C]-N-acetoxy-2-acetylaminofluorene in terms of alterations in DNA **secondary structure**, ability to reconstitute nucleosome structures in chromatin, and template activity for in vitro transcription. In contrast to the control native DNA, the carcinogen-modified DNA was susceptible to partial digestion by the single-strand-specific endonuclease S1. Depending on the particular conditions, for every [14C]-N-2-acetylaminofluorene residue released, about

5 to 35 base pairs of DNA were also released during the S1 nuclease digestion. Chromatin was reconstituted in vitro utilizing [14C]-N-2-acetylaminofluorene-modified DNA and unmodified chromatin-associa ted proteins. This reconstituted chromatin showed the same kinetics and extent of digestion by staphylococcal nuclease and similar nucleosome profiles on sucrose gradient density centrifugation as those obtained with native chromatin or chromatin reconstituted with unmodified DNA. The carcinogen-modified DNA and also chromatin reconstituted from this DNA showed, however, marked **reductions** in their abilities to serve as templates for transcription with Escherichia coli **RNA polymerase** . These results suggest that the covalent binding of N-2-acetylaminofluorene to DNA produces localized regions of denaturation in the DNA and that this is associated with a marked impairment in template activity during transcription. This modification, however, does not grossly affect the ability of the DNA to interact with chromosomal proteins to form apparently normal nucleosome structures.

4/3,AB/101 (Item 101 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02190126 76039930 PMID: 1101955
T7 **RNA polymerase** : conformation, functional groups, and promotor binding.
Oakley J L; Pascale J A; Coleman J E
Biochemistry (UNITED STATES) Oct 21 1975, 14 (21) p4684-91, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Circular dichroic spectra of T7 **RNA polymerase** show minima at 222 nm ([theta]m=-7.9 X 10(3) deg cm2/dmol) and 208 nm ([theta]m =-7.55 X 10(3) deg cm2/dmol) and a maximum at 193 nm ([theta]m = 1.2 X 10(4) deg cm2/dmol). The small mean residue ellipticity above 200 nm indicates that the **secondary structure** contains approximately 12% alpha helix. The **secondary structure** is unaltered by high salt, glycerol, -SH reagents, nitration of tyrosyl residues, and chelating agents. Binding of the native enzyme to [32P]T7 DNA has been measured by the retention of the protein-[32P]DNA complexes on nitrocellulose filters. At 37degrees T7 **RNA polymerase** binds to its promoters in the absence of NTP's. Binding and catalytic activity are both abolished at 0degree. Binding of the initiating [gamma-32P]GTP can also be detected by the filter binding assay. Native T7 **RNA polymerase** is inactivated by reaction with 1 mol of 5,5'-dithiobis(2-nitrobenzoic acid) (Nbs2) or 1 mol of [14C]iodoacetamide. The latter reaction is blocked by Nbs2 suggesting that a single -SH group is required for activity. Alkylation of the -SH group does not alter binding of the enzyme to the DNA template, but modifies the binding of GTP to the enzyme. Nitration of approximately4 surface tyrosyl residues of the protein prevents binding to T7 DNA. The restriction endonuclease, Hpa II, cuts T7 DNA into approximately40 fragments and **reduces** total **RNA** synthesis by T7 **RNA polymerase** by 70%. Fragmentation of the DNA template by Hpa II does not alter the rate of **RNA** chain initiation by T7 **polymerase** , and restriction fragments accounting for approximately25% of the T7 DNA still bind tightly to the enzyme. Thus the T7 **RNA polymerase** promoters remain intact on the restriction fragments. Gel electrophoresis of the transcription products, using restriction fragments as templates, show that of the seven in vitro transcripts produced by T7 **RNA polymerase** from whole T7 DNA, only the smallest (representing the last 1.5% of the genome) is transcribed from Hpa II fragments. The remaining transcripts are replaced by six new and much shorter mRNA's. The DNA fragments containing the promoters for these mRNA's have been removed from the fragment mix by binding them to the enzyme and retaining the complexes on nitrocellulose

filters.

4/3,AB/102 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13923250 BIOSIS NO.: 200200552071
Effects of breed, parity, and folic acid supplement on the expression of folate metabolism genes in endometrial and embryonic tissues from sows in early pregnancy.
AUTHOR: Vallee Maud; Guay Frederic; Beaudry Daniele; Matte Jacques; Blouin Richard; Laforest Jean-Paul; Lessard Martin; Palin Marie-France(a)
AUTHOR ADDRESS: (a)Agriculture and Agri-Food Canada, 2000 Route 108 East, P.O. Box 90, Lennoxville, QC, J1M 1Z3**Canada E-Mail: palinmf@agr.gc.ca
JOURNAL: Biology of Reproduction 67 (4):p1259-1267 October, 2002
MEDIUM: print
ISSN: 0006-3363
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Folic acid and glycine are factors of great importance in early gestation. In sows, folic acid supplement can increase litter size through a decrease in embryonic mortality, while glycine, the most abundant amino acid in the sow oviduct, uterine, and allantoic fluids, is reported to act as an organic osmoregulator. In this study, we report the characterization of cytoplasmic serine hydroxymethyltransferase (cSHMT), T-protein, and vT-protein (variant T-protein) mRNA expression levels in endometrial and embryonic tissues in gestating sows on Day 25 of gestation according to the breed, parity, and folic acid + glycine supplementation. Expression levels of cSHMT, T-protein, and vT-protein mRNA in endometrial and embryonic tissues were performed using semiquantitative reverse transcription-**polymerase** chain reaction. We also report, for the first time, an alternative splicing event in the porcine T-protein gene. Results showed that a T-protein splice variant, vT-protein, is present in all the tested sow populations. Further characterizations revealed that this T-protein splice variant contains a coding intron that can adopt a **secondary structure**. Results demonstrated that cSHMT mRNA expression levels were significantly higher in sows receiving the folic acid + glycine supplementation, independently of the breed or parity and in both endometrial and embryonic tissues. Upon receiving the same treatment, the vT-protein and T-protein mRNA expression levels were significantly **reduced** in the endometrial tissue of Yorkshire-Landrace sows only. These results indicate that modulation of specific gene expression levels in endometrial and embryonic tissues of sows in early gestation could be one of the mechanism involved with the role of folic acid on improving swine reproduction traits.
2002

4/3,AB/103 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13888870 BIOSIS NO.: 200200517691
Knock-out of a putative transporter results in altered blue-light signalling in Chlamydomonas.
AUTHOR: Dame Gregory; Gloeckner Gernot; Beck Christoph F(a)
AUTHOR ADDRESS: (a)Institut fuer Biologie III, Universitaet Freiburg, Schaenzlestrasse 1, D-79104, Freiburg**Germany E-Mail: beck@uni-freiburg.de
JOURNAL: Plant Journal 31 (5):p577-587 September, 2002

MEDIUM: print
ISSN: 0960-7412
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Nitrogen starvation and blue light are the two environmental cues that control sexual differentiation in Chlamydomonas reinhardtii. Insertional mutagenesis was applied to generate mutants that still require nitrogen starvation as the initiating signal for gametogenesis but were no longer dependent on irradiation. In one mutant analysed, sequences adjacent to the site of insertion were cloned and used for the isolation of a genomic clone that, upon transformation, could complement the mutant phenotype. The gene identified (LRG6) encodes two mRNAs that appear to be the products of differential splicing. The two putative gene products derived from these mRNAs differ in their C-terminal ends. Both predicted gene products exhibit multiple hydrophobic domains with alpha-helical **secondary structure** typical for integral membrane proteins. These proteins may form pores, and may function as transporters of as-yet unknown substrates. Since rendering the LRG6 gene non-functional resulted in light-independence of gamete formation, it is suggested that this transporter may inhibit signal flux from the photoreceptor to target genes - either directly by its activity or indirectly by serving as a scaffold for signalling proteins. Shutting off this transporter may be required for the activation of signal flux in this pathway. This concept is supported by the observed **reduction** in LRG6 mRNA levels during the first phase of gametic differentiation.

2002

4/3,AB/104 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13817048 BIOSIS NO.: 200200445869
A RAC protein-binding site in the internal transcribed spacer 2 of pre-rRNA transcripts from Schizosaccharomyces pombe.
AUTHOR: Abeyrathne Priyanka D; Lalev Atanas I; Nazar Ross N(a)
AUTHOR ADDRESS: (a)Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, N1G 2W1**Canada E-Mail: rnnazar@UoGuelph.CA
JOURNAL: Journal of Biological Chemistry 277 (24):p21291-21299 June 14, 2002
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The interdependence of steps in the processing of the eukaryotic preribosomal rRNA transcripts indicate that rRNA processing, at least in part, acts as a quality control mechanism to help ensure that only functional rRNA is incorporated into mature ribosomes. In search of structural components that underlie this interdependence, we have isolated a large protein complex or RAC that contains an independent binding site for all four of the transcribed spacers in the nascent pre-rRNA. In this study the RAC-binding site in the internal transcribed spacer 2 sequence of Schizosaccharomyces pombe rRNA transcripts was identified, and the influence of this site on rRNA maturation was assessed. Modification exclusion analyses indicate that the protein complex interacts with a helical domain previously shown to contain features common to both the internal transcribed spacer 1 and the 3'-external transcribed spacer. Mutagenic analyses in vitro confirm an interaction with this sequence, and parallel analyses in vivo indicated a

critical role in both the maturation of the rRNA components of the large subunit as well as the 18 S rRNA component of the small subunit. Hybridization analyses also indicated greatly elevated levels of unprocessed nascent **RNA**. These effects are contrasted with mutations in other regions of the **secondary structure** that resulted in some **reduction** of plasmid-derived mature rRNA but no elevated levels of the precursor molecules. The significance with respect to rRNA maturation and the interdependences in rRNA processing are discussed.

2002

4/3,AB/105 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13747607 BIOSIS NO.: 200200376428
Purification and characterization of the mammaglobin/lipophilin B complex, a promising diagnostic marker for breast cancer.
AUTHOR: Carter Darrick(a); Douglass John F; Cornellison Charisa D; Retter Marc W; Johnson Jeffrey C; Bennington Angela A; Fleming Timothy P; Reed Steven G; Houghton Raymond L; Diamond Deborah L; Vedvick Thomas S
AUTHOR ADDRESS: (a)Department of Antigen Discovery, Corixa Corp., 1124 Columbia Street, Suite 200, Seattle, WA, 98104**USA E-Mail: carter@corixa.com
JOURNAL: Biochemistry 41 (21):p6714-6722 May 28, 2002
MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Mammaglobin, a promising diagnostic marker for breast cancer, forms a covalent complex with lipophilin B. mRNA levels for each component of the complex were determined for a number of breast tumors and normal tissues, and correlation of message expression was highly significant between mammaglobin and lipophilin B ($p < 0.0001$). The complex was purified by both standard biochemical techniques and immunoaffinity chromatography. N-Terminal sequencing revealed that mammaglobin and lipophilin B are processed as predicted by cleavage of their signal sequence after amino acids 19 and 21, respectively. Three molecular masses-representing the fully glycosylated form, the complex without one of the carbohydrate chains, and the deglycosylated proteins-are detected by ProteinChip array SELDI-TOF mass spectrometry after partial enzymatic deglycosylation. This is consistent with the two predicted N-linked glycosylation sites in the primary sequence of mammaglobin and each site having an attached sugar of apprx3500 Da. **Reducing** agents release lipophilin B from mammaglobin, and the free peptides are seen at their predicted molecular masses in the deglycosylated complex. Molecular modeling, **secondary structure** prediction, and circular dichroism indicate that the complex is a small alpha-helical globule that has three disulfide bridges and a carbohydrate chain at each pole. LC-ESI-MS shows that mammaglobin and lipophilin B are bonded in a head to tail orientation. This work describes the biochemistry of the mammaglobin/lipophilin B complex and lays the framework for use of this complex as a novel protein-based serological marker for breast cancer.

2002

4/3,AB/106 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13697298 BIOSIS NO.: 200200326119
Nontemplated nucleotide addition by HIV-1 reverse transcriptase.
AUTHOR: Golinelli Marie-Pierre; Hughes Stephen H(a)
AUTHOR ADDRESS: (a)HIV Drug Resistance Program, National Cancer Institute at Frederick, Building 539, Room 130A, Frederick, MD, 21702-1201**USA
E-Mail: hughes@ncifcrf.gov
JOURNAL: Biochemistry 41 (18):p5894-5906 May 7, 2002
MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We studied the kinetics of nontemplated nucleotide addition by the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) using model substrates derived from the 3' end of HIV-1 minus-strand strong-stop DNA. The addition of a nontemplated nucleotide was highly dependent on the nature of the base (fastest addition with dATP), type of nucleoside, and pH of the reaction buffer. The salt concentration, presence or absence of nucleocapsid protein, and nature of the blunt-ended duplex (DNA/DNA versus **RNA**/DNA) had only limited effects. The efficiency and base specificity were strongly affected by the sequence at the 3' end of the blunt-ended duplex. In every case, nontemplated nucleotide addition was much slower than templated polymerization. The Kd for the incoming dNTP with an RT bound to a blunt-ended duplex was at least 1000-fold higher than with a duplex with a template overhang. At concentrations normally found in vivo, ATP can compete with dNTPs for binding to the **polymerase** active site and **reduce** the efficiency of nontemplated nucleotide addition. Although a stable ternary complex RT/DNA/dNTP could be readily detected by gel retardation assays if the DNA had a template overhang, stable ternary complexes were not observed with a blunt-ended duplex substrate. At in vivo concentrations of dNTPs (5-10 muM), nontemplated nucleotide addition occurred, but it was very inefficient and the rate of nontemplated polymerization is at least 10000-fold slower than the rate of templated polymerization. We could conclude that, in vivo, the unfavorable binding of the incoming dNTP, low concentration of dNTPs, the presence of a large concentration of ATP, and the inability to form a stable ternary complex prior to the polymerization step collaborate to **reduce** the efficiency of nontemplated nucleotide addition.

2002

4/3,AB/107 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13519082 BIOSIS NO.: 200200147903
Reduction of wobble-position GC bases in Corynebacteria genes and enhancement of PCR and heterologous expression.
AUTHOR: Sanli Gulsah; Blaber Sachiko I; Blaber Michael(a)
AUTHOR ADDRESS: (a)Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, 32306-3015**USA
E-Mail: blaber@sb.fsu.edu
JOURNAL: Journal of Molecular Microbiology and Biotechnology 3 (1):p 123-126 January, 2001
MEDIUM: print
ISSN: 1464-1801
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Corynebacteria codon usage exhibits an overall GC content of 67%, and a wobble-position GC content of 88%. Escherichia coli, on the other hand has an overall GC content of 51%, and a wobble-position GC content of 55%. The high GC content of Corynebacteria genes results in an unfavorable codon preference for heterologous expression, and can present difficulties for **polymerase**-based manipulations due to **secondary-structure** effects. Since these characteristics are due primarily to base composition at the wobble-position, synthetic genes can, in principle, be designed to eliminate these problems and retain the wild-type amino acid sequence. Such genes would obviate the need for special additives or bases during in vitro **polymerase**-based manipulation and mutant host strains containing uncommon tRNA's for heterologous expression. We have evaluated synthetic genes with **reduced** wobble-position G/C content using two variants of the enzyme 2,5-diketo-D-gluconic acid **reductase** (2,5-DKGR A and B) from Corynebacterium. The wild-type genes are refractory to **polymerase** -based manipulations and exhibit poor heterologous expression in enteric bacteria. The results indicate that a subset of codons for five amino acids (alanine, arginine, glutamate, glycine and valine) contribute the greatest contribution to **reduction** in G/C content at the wobble-position. Furthermore, changes in codons for two amino acids (leucine and proline) enhance bias for expression in enteric bacteria without affecting the overall G/C content. The synthetic genes are readily amplified using **polymerase**-based methodologies, and exhibit high levels of heterologous expression in E. coli.

2001

4/3,AB/108 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12979601 BIOSIS NO.: 200100186750
Characterization of **RNA** aptamer binding by the Wilms' tumor suppressor protein WT1.
AUTHOR: Zhai Gary; Iskandar Maya; Barilla Kathleen; Romaniuk Paul J(a)
AUTHOR ADDRESS: (a)Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8W 3P6: pjr@uvic.ca**Canada
JOURNAL: Biochemistry 40 (7):p2032-2040 February 20, 2001
MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The interaction of the zinc finger protein WT1 with **RNA** aptamers has been investigated using a quantitative binding assay, and the results have been compared to those from a previous study of the DNA binding properties of this protein. A recombinant peptide containing the four zinc fingers of WT1 (WT1-ZFP) binds to representatives of three specific families of **RNA** aptamers with apparent dissociation constants ranging from 13.8 +- 1.1 to 87.4 +- 10.4 nM, somewhat higher than the dissociation constant of 4.12 +- 0.4 nM for binding to DNA. An isoform that contains an insertion of three amino acids between the third and fourth zinc fingers (WT1 (+KTS)-ZFP) also binds to these RNAs with slightly **reduced** affinity (the apparent dissociation constants ranging from 22.8 to 69.8 nM) but does not bind to DNA. The equilibrium binding of WT1-ZFP to the highest-affinity **RNA** molecule was compared to the equilibrium binding to a consensus DNA molecule as a function of temperature, pH, monovalent salt concentration, and divalent salt concentration. The interaction of WT1-ZFP with both nucleic acids is

an entropy-driven process. Binding of WT1-ZFP to **RNA** has a pH optimum that is narrower than that observed for binding to DNA. Binding of WT1-ZFP to DNA is optimal at 5 mM MgCl2, while the highest affinity for **RNA** was observed in the absence of MgCl2. Binding of WT1 to both nucleic acid ligands is sensitive to increasing monovalent salt concentration, with a greater effect observed for DNA than for **RNA**. Point mutations in the zinc fingers associated with Denys-Drash syndrome have dramatically different effects on the interaction of WT1-ZFP with DNA, but a consistent and modest effect on the interaction with **RNA** . The role of **RNA** sequence and **secondary structure** in the binding of WT1-ZFP was probed by site-directed mutagenesis. Results indicate that a hairpin loop is a critical structural feature required for protein binding, and that some consensus nucleotides can be substituted provided proper base pairing of the stem of the hairpin loop is maintained.

2001

4/3,AB/109 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12944372 BIOSIS NO.: 200100151521
An **RNA** domain within the 5' untranslated region of the tomato bushy stunt virus genome modulates viral **RNA** replication.
AUTHOR: Wu Baodong; Vanti William B; White K Andrew(a)
AUTHOR ADDRESS: (a)Department of Biology, York University, Toronto, ON, M3J 1P3: kawhite@yorku.ca**Canada
JOURNAL: Journal of Molecular Biology 305 (4):p741-756 26 January, 2001
MEDIUM: print
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The terminal half of the 5' untranslated region (UTR) in the (+)-strand **RNA** genome of tomato bushy stunt virus was analyzed for possible roles in viral **RNA** replication. Computer-aided thermodynamic analysis of **secondary structure**, phylogenetic comparisons for base-pair covariation, and chemical and enzymatic solution **structure** probing were used to analyze the 78 nucleotide long 5'-terminal sequence. The results indicate that this sequence adopts a branched **secondary structure** containing a three-helix junction core. The T-shaped domain (TSD) formed by this terminal sequence is closed by a prominent ten base-pair long helix, termed stem 1 (S1). Deletion of either the 5' or 3' segment forming S1 (coordinates 1-10 or 69-78, respectively) in a model subviral **RNA** replicon, i.e. a prototypical defective interfering (DI) **RNA**, **reduced** in vivo accumulation levels of this molecule approximately 20-fold. Compensatory-type mutational analysis of S1 within this replicon revealed a strong correlation between formation of the predicted S1 **structure** and efficient DI **RNA** accumulation. **RNA** decay studies in vivo did not reveal any notable changes in the physical stabilities of DI RNAs containing disrupted S1s, thus implicating **RNA** replication as the affected process. Further investigation revealed that destabilization of S1 in the (+)-strand was significantly more detrimental to DI **RNA** accumulation than (-)-strand destabilization, therefore S1-mediated activity likely functions primarily via the (+)-strand. The essential role of S1 in DI **RNA** accumulation prompted us to examine the 5'-proximal **secondary structure** of a previously identified mutant DI **RNA**, **RNA** B, that lacks the 5' UTR but is still capable of low levels of

replication. Mutational analysis of a predicted S1-like element present within a cryptic 5'-terminal TSD confirmed the importance of the former in **RNA** B accumulation. Collectively, these data support a fundamental role for the TSD, and in particular its S1 sub-element, in tombusvirus **RNA** replication.

2001

4/3,AB/110 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12710710 BIOSIS NO.: 200000464212
Diabetes-induced myocardial structural changes: Role of endothelin-1 and its receptors.
AUTHOR: Chen Shali; Evans Terry; Mukherjee Kallol; Karmazyn Morris; Chakrabarti Subrata(a)
AUTHOR ADDRESS: (a)Department of Pathology, University of Western Ontario, London, ON, N6A 5A5**Canada
JOURNAL: Journal of Molecular and Cellular Cardiology 32 (9):p1621-1629 September, 2000
MEDIUM: print
ISSN: 0022-2828
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Several metabolic abnormalities may be triggered **secondary** to hyperglycemia in diabetes. Some of these abnormalities may alter expression of vasoactive factors in the target organs of diabetic complications. We investigated alterations of endothelin-1 (ET-1) and its receptors, ETA and ETB, and associated structural changes in the myocardium of streptozotocin-induced diabetic rats after 6 months of hyperglycemia. We further assessed the preventive effects of an ET-receptor antagonist bosentanTM on these changes. Compared to the non-diabetic, age- and sex-matched control animals, diabetic rats showed hyperglycemia, glucosuria, **reduced** body weight gain and elevated glycated Hb levels. Measurement of ET-1, ETA and ETB mRNAs by semiquantitative RT-PCR showed significantly increased mRNA levels in the hearts of diabetic rats. Treatment with bosentanTM failed to **reduce** ET-1 or ETB mRNA expression in diabetes, however ETA mRNA expression was **reduced**. Immunocytochemically, ET-1 was detected in the cardiomyocytes, endothelium and smooth muscle cells of the larger blood vessels and was increased in diabetes. Autoradiographic localization of ET-1 receptors, using 125I-ET-1, showed increased binding in the endothelium and myocardium of diabetic animals. Histologically, focal fibrous scarring with apoptotic cardiomyocytes, consistent with changes **secondary** to microvascular occlusion, was only present in the diabetic rats. In keeping with focal fibrosis, myocardium from diabetic rats further showed significantly increased mRNA expression of two extracellular matrix protein transcripts, fibronectin and collagen alpha1(IV) which were completely prevented by treatment with bosentanTM. These data suggest that hyperglycemia-induced upregulation of the ET-system in the heart may be important in the pathogenesis of cardiac involvement in diabetes.

2000

4/3,AB/111 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12359771 BIOSIS NO.: 200000113273
The kinetic mechanism of the hairpin ribozyme in vivo: Influence of **RNA** helix stability on intracellular cleavage kinetics.
AUTHOR: Donahue Christine P; Yadava Ramesh S; Nesbitt Steven M; Fedor Martha J(a)
AUTHOR ADDRESS: (a)Department of Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd, MB35, La Jolla, CA, 92037**USA
JOURNAL: Journal of Molecular Biology 295 (3):p693-707 Jan. 21, 2000
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The relationship between hairpin ribozyme **structure**, and cleavage and ligation kinetics, and equilibria has been characterized extensively under a variety of reaction conditions in vitro. We developed a quantitative assay of hairpin ribozyme cleavage activity in yeast to learn how **structure**-function relationships defined for **RNA** enzymes in vitro relate to **RNA**-mediated reactions in cells. Here, we report the effects of variation in the stability of an essential **secondary structure** element, H1, on intracellular cleavage kinetics. H1 is the base-paired helix formed between ribozyme and 3' cleavage product RNAs. H1 sequences with fewer than three base-pairs fail to support full activity in vitro or in vivo, arguing against any significant difference in the stability of short **RNA** helices under in vitro and intracellular conditions. Under standard conditions in vitro that include 10 mM MgCl2, the internal equilibrium between cleavage and ligation of ribozyme-bound products favors ligation. Consequently, ribozymes with stable H1 sequences display sharply **reduced** self-cleavage rates, because cleavage is reversed by rapid re-ligation of bound products. In contrast, ribozymes with as many as 26 base-pairs in H1 continue to self-cleave at maximum rates in vivo. The failure of large products to inhibit cleavage could be explained if intracellular conditions promote rapid product dissociation or shift the internal equilibrium to favor cleavage. Model experiments in vitro suggest that the internal equilibrium between cleavage and ligation of bound products is likely to favor cleavage under intracellular ionic conditions.

2000

4/3,AB/112 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12350329 BIOSIS NO.: 200000103831
Structural and functional analysis of the 5' untranslated region of coxsackievirus B3 **RNA**: In vivo translational and infectivity studies of full-length mutants.
AUTHOR: Liu Zhewei; Carthy Christopher M; Cheung Paul; Bohunek Lubos; Wilson Janet E; McManus Bruce M; Yang Decheng(a)
AUTHOR ADDRESS: (a)Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, V6Z 1Y6**Canada
JOURNAL: Virology 265 (2):p206-217 Dec. 20, 1999
ISSN: 0042-6822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The lengthy 5' untranslated region (5'UTR) of coxsackievirus B3

(CVB3) forms a highly ordered **secondary structure**, which plays an important role in controlling viral transcription and translation. Our previous work has delineated the internal ribosome entry site (IRES) by mutation of mono- and bicistronic plasmids containing the 5'UTR and subsequent cell- free translation in rabbit reticular lysate (D. Yang, J. E. Wilson, D. R. Anderson, L. Bohunek, C. Cordeiro, R. Kandolf, and B. M. McManus. (1997). Virology 228, 63-73). To further identify the sequence elements responsible for viral translation and infectivity in tissue culture cells, >30 full-length mutants of CVB3 were constructed by mutations of the IRES and its flanking regions. Viral RNAs were transcribed from these constructs and transfected into HeLa cells. When the stem-loops G and H in the putative IRES were deleted, viral infectivity was abolished and viral protein translation was also undetectable by immunoblot analysis. However, when stem-loops A and B were deleted or stem-loop E was partially deleted, viral protein translation could be detected although cytopathic effect could not be observed. The data suggest that the crucial sequence of the IRES is located at stem-loops G and H. Further serial deletion mapping up and down stream of the crucial sequence defined more accurately the 5' and 3' boundaries of the IRES, located at nucleotides (nts) 309-432 and 639-670, respectively. These results indicate that the core sequence of the IRES should be located at nts 432-639. This IRES segment is much shorter and located closer to the initiation codon than that of poliovirus. To further define critical nucleotides within the IRES core, site-directed mutagenesis was conducted at the IRES core sequence by PCR. A 46-nt deletion in the pyrimidine-rich tract of stem-loop G abolished viral translation and infectivity. Interestingly, five single-nt substitutions in the pyrimidine-rich tract aimed at destabilizing the base pairing between the viral IRES and host 18S rRNA did not abolish CVB3 infectivity although viral protein translation was significantly **reduced**. This finding suggests that ribosomal internal initiation of translation and viral infectivity not only may require **RNA secondary structure** but also may need tertiary **structure** and perhaps the assistance of host protein factors.

1999

4/3,AB/113 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12350048 BIOSIS NO.: 200000103550
Effect of mRNA **secondary structure** in the regulation of gene expression: Unfolding of stable loop causes the expression of Taq **polymerase** in E. coli.
AUTHOR: Mukund Mandayam Alasingachar; Bannerjee Tanmay; Ghosh Indira; Datta Santanu(a)
AUTHOR ADDRESS: (a)Astra Research Centre India, 18th Cross, Malleswaram, Bangalore, 560 003**India
JOURNAL: Current Science (Bangalore) 76 (11):p1486-1490 June 10, 1999
ISSN: 0011-3891
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The presence of **secondary structure** in the 5' region of a mRNA modulates the translational efficiency in E. coli. A computer program 'ANARCI' which enables us to study the propensity of any single stranded nucleotide sequence to form stable **secondary** structures has been developed. A unique feature of this program is a mutation module which allows simulation of a gradual unfolding of a **secondary structure** by changing the nucleotide sequence in a manner such that

the corresponding free energy is **reduced** while the coding amino acid sequence remains unchanged. We have analysed the 5' region of a number of E. coli mRNAs. The results indicated that highly expressed genes had unfolded 5' region in their mRNA whereas some of the poorly expressed genes had stable fold with DELTAG < -5.5 kcal/mole. In order to determine the effect of stable fold on the heterologous expression of genes in E. coli we took the test case of Taq **polymerase**. The wild-type gene of Taq **polymerase** was poorly expressed in E. coli and had a stable fold in 5' coding region including the initiation codon AUG with a DELTAG = -9.8 kcal/mole. This stable fold presumably interferes with the interaction of the initiation AUG codon, with the f-Met-tRNA, thus arresting translation. Using ANARCI we could predict point mutations in the fold region (without changing the amino acid sequence) which would break the predicted folded **structure**. The gene carrying the designed point mutations was constructed and tested for expression of Taq **polymerase**. A 24-fold increase in the level of expression of the enzyme was obtained.

1999

4/3,AB/114 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12303249 BIOSIS NO.: 200000061116
Phylogenetic analysis of the apolipoprotein B mRNA-editing region. Evidence for a **secondary structure** between the mooring sequence and the 3' efficiency element.
AUTHOR: Hersberger Martin; Patarroyo-White Susannah; Arnold Kay S; Innerarity Thomas L(a)
AUTHOR ADDRESS: (a)Gladstone Institute of Cardiovascular Disease, San Francisco, CA**USA
JOURNAL: Journal of Biological Chemistry 274 (49):p34590-34597 Dec. 3, 1999
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Apolipoprotein (apo) B mRNA editing is the deamination of C6666 to uridine, which changes the codon at position 2153 from a genomically encoded glutamine (CAA) to an in-frame stop codon (UAA). The apoB mRNA-editing enzyme complex recognizes the editing region of the apoB pre-mRNA with exquisite precision. Four sequence elements spanning 139 nucleotides (nt) on the apoB mRNA have been identified that specify this precision. In cooperation with the indispensable mooring sequence and spacer element, a 5' efficiency element and a 3' efficiency element enhance editing in vitro. A phylogenetic comparison of 32 species showed minor differences in the apoB mRNA sequence, and the apoB mRNA from 31 species was robustly edited in vitro. However, guinea pig mRNA was poorly edited. Compared with the consensus sequences of these 31 species, guinea pig apoB mRNA has three variations in the 3' efficiency element, and the conversion of these to the consensus sequence increased editing to the levels in the other species. From this information, a model for the **secondary structure** was formulated in which the mooring sequence and the 3' efficiency element form a double-stranded stem. Thirty-one mammalian apoB mRNA sequences are predicted to form this stem positioning C6666 two nucleotides upstream of the stem. However, the guinea pig apoB mRNA has a mutation in the 3' efficiency element (C6743 to U) that predicts an extension of the stem and hence the lower editing efficiency. A test of this model demonstrated that a single substitution at 6743 (U to C) in the guinea pig apoB mRNA, that should **reduce**

the stem, enhanced editing, and mutations in the 3' efficiency element that extended the stem for three base pairs dramatically **reduced** editing. Furthermore, the addition of a 20-nucleotide 3' efficiency element **RNA**, to a 58-nucleotide guinea pig apoB mRNA lacking the 3' efficiency element more than doubled the in vitro editing activity. Based on these results, a model is proposed in which the mooring sequence and the 3' efficiency element form a double-stranded stem, thus suggesting a mechanism of how the 3' efficiency element enhances editing.

1999

4/3,AB/115 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12008066 BIOSIS NO.: 199900288585
Molecular diversity of arbuscular mycorrhizal fungi colonising Hyacinthoides non-scripta (bluebell) in a seminatural woodland.
AUTHOR: Helgason T(a); Fitter A H; Young J P W
AUTHOR ADDRESS: (a)Department of Biology, University of York, York, YO10 5YW**UK
JOURNAL: Molecular Ecology 8 (4):p659-666 April, 1999
ISSN: 0962-1083
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Arbuscular mycorrhizal (AM) fungi form symbiotic associations with plant roots. Around 150 species have been described and it is becoming clear that many of these species have different functional properties. The species diversity of AM fungi actively growing in roots is therefore an important component of ecosystem diversity. However, it is difficult to identify AM fungi below the genus level from morphology in planta, as they possess few informative characters. We present here a molecular method for identifying infra-generic sequence types that estimate the taxonomic diversity of AM fungi present in actively growing roots. Bluebell roots were sampled from beneath two different canopy types, oak and sycamore, and DNA sequences were amplified from roots by the **polymerase** chain reaction with fungal-specific primers for part of the small subunit ribosomal **RNA** gene. Restriction fragment length polymorphism among 141 clones was assessed and 62 clones were sequenced. When aligned, discrete sequence groups emerged that cluster into the three families of AM fungi: Acaulosporaceae, Gigasporaceae and Glomaceae. The sequence variation is consistent with rRNA **secondary structure**. The same sequence types were found at both sampling times. Frequencies of Scutellospora increased in December, and Acaulospora increased in abundance in July. Sites with a sycamore canopy show a **reduced** abundance of Acaulospora, and those with oak showed a **reduced** abundance of Glomus. These distribution patterns are consistent with previous morphological studies carried out in this woodland. The molecular method provides an alternative method of estimating the distribution and abundance of AM fungi, and has the potential to provide greater resolution at the infrageneric level.

1999

4/3,AB/116 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11446051 BIOSIS NO.: 199800227383

Translationally repressive **RNA** structures monitored in vivo using temperate DNA bacteriophages.
AUTHOR: Fouts Derrick E; Celander Daniel W(a)
AUTHOR ADDRESS: (a)B103 Chemical Life Sci. lab., 601 South Goodwin Ave., Urbana, IL 61801**USA
JOURNAL: Gene (Amsterdam) 210 (1):p135-142 March 27, 1998
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The **RNA** challenge phage system enables genetic selection of proteins with **RNA**-binding activity in bacteria. These phages are modified versions of the temperate DNA bacteriophage P22 in which post-transcriptional regulatory events control the developmental fate of the phage. The system was originally developed to identify novel **RNA** ligands that display **reduced** affinity for the R17/MS2 coat protein, as well as to select for suppressor coat proteins that recognize mutant **RNA** ligands. During the course of evaluating whether the HIV-1 Rev protein could direct lysogen development for bacteriophage derivatives that encode Rev response element (RRE) **RNA** sequences, two examples of RRE **RNA** ligands that interfere with challenge phage development were identified. In the phage examples described, RRE **RNA secondary structure** prevents Ant protein biosynthesis and lytic development. Phage lysogen formation occurs efficiently in recipient cells, independent of the expression status of the Rev protein or trans-acting competitor RRE **RNA** ligands. These studies provide the first example whereby **RNA** challenge phages may be applied to study **RNA** folding events and **RNA** structural interactions in an in vivo context.

1998

4/3,AB/117 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10383963 BIOSIS NO.: 199699005108
The **RNA**-binding protein HF-I, known as a host factor for phage Q-beta **RNA** replication, is essential for rpoS translation in Escherichia coli.
AUTHOR: Muffler Andrea; Fischer Daniela; Hengge-Aronis Regine(a)
AUTHOR ADDRESS: (a)Dep. Biology, University Konstanz, 78434 Konstanz** Germany
JOURNAL: Genes & Development 10 (9):p1143-1151 1996
ISSN: 0890-9369
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The rpoS-encoded sigma-S subunit of **RNA polymerase** in Escherichia coli is a global regulatory factor involved in several stress responses. Mainly because of increased rpoS translation and stabilization of sigma-S, which in nonstressed cells is a highly unstable protein, the cellular sigma-S content increases during entry into stationary phase and in response to hyperosmolarity. Here, we identify the hfq-encoded **RNA**-binding protein HF-I, which has been known previously only as a host factor for the replication of phage Q-beta **RNA**, as an essential factor for rpoS translation. An hfq null mutant exhibits strongly **reduced** sigma-S levels under all conditions tested and is deficient for growth phase-related and osmotic induction of us. Using a combination of gene fusion analysis and pulse-chase experiments, we demonstrate that the hfq mutant is specifically impaired in rpoS

translation. We also present evidence that the H-NS protein, which has been shown to affect zpoS translation, acts in the same regulatory pathway as HF-I at a position upstream of HF-I or in conjunction with HF-I. In addition, we show that expression and heat induction of the heat shock sigma factor sigma-32 (encoded by zpoH) is not dependent on HF-I, although rpoH and rpoS are both subject to translational regulation probably mediated by changes in mRNA **secondary structure**. HF-I is the first factor known to be specifically involved in rpoS translation, and this role is the first cellular function to be identified for this abundant ribosome-associated **RNA**-binding protein in E. coli.

1996

4/3,AB/118 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09996345 BIOSIS NO.: 199598451263
Mutation analysis of the role of the first helix of region 4.2 of the sigma-70 subunit of Escherichia coli **RNA polymerase** in transcriptional activation by activator protein PhoB.
AUTHOR: Kim Soo-Ki; Makino Kozo(a); Amemura Mitsuko; Nakata Atsuo; Shinagawa Hideo
AUTHOR ADDRESS: (a)Dep. Molecular Microbiol., Res. Inst. Microbial Dis., Osaka Univ., 3-1 Yamadaoka, Suita, Osaka 5**Japan
JOURNAL: Molecular & General Genetics 248 (1):p1-8 1995
ISSN: 0026-8925
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Transcription of the genes belonging to the phosphate (pho) regulon in Escherichia coli requires the specific activator protein PhoB, in addition to **RNA polymerase** containing the major sigma factor, sigma-70 which is encoded by rpoD. We previously isolated two mutant sigma-70s (D570G and E575K) that were specifically defective in transcribing the pho genes. The mutated sites were located near and within the first helix of the helix-turn-helix (HTH) motif of region 4.2 of sigma-70. To study further the role of the first helix of the HTH motif of sigma-70 in transcriptional activation by PhoB, we made a series of rpoD mutations that alter the motif and purified the mutant sigma-70 proteins. **RNA** polymerases containing the mutant sigma-70s Y571A, T572L, V576T, K578E and F580V showed **reduced** in vitro transcription from the pstS promoter, a representative pho promoter, in the presence of PhoB, whereas **RNA polymerase** containing another mutant sigma-70 (E574 K) showed enhanced transcription from the promoter. Transcription from the activator-independent tac promoter and the pBR-P4 promoter, which is independent of PhoB and requires cAMP-CRP (cAMP receptor protein) for transcription, was affected at most only marginally by these sigma-701 70 mutations. These results provide further evidence that the first helix plays an important role in the specific interaction between **RNA polymerase** and PhoB protein bound to the pho promoters in transcriptional activation.

1995

4/3,AB/119 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09284139 BIOSIS NO.: 199497292509

The proximate 5' and 3' ends of the 120-base viral **RNA** (pRNA) are crucial for the packaging of bacteriophage vphi-29 DNA.
AUTHOR: Zhang Chunlin; Lee Choong-Sik; Guo Peixuan(a)
AUTHOR ADDRESS: (a)Purdue Cancer Cent., B-36 Hansen Life Sci. Res. Build., Purdue Univ., West Lafayette, IN 47907**USA
JOURNAL: Virology 201 (1):p77-85 1994
ISSN: 0042-6822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In vitro mutagenesis was performed to identify the DNA packaging domain of the 120-base pRNA essential and specific for DNA encapsidation by bacteriophage vphi-29 of Bacillus subtilis. All deletions and mutations targeted the 5' and 3' ends of the pRNA. DNA templates of a control or mutant pRNAs used for in vitro transcription with T7 **RNA polymerase** were generated by PCR. Fourteen mutant pRNA molecules were synthesized from DNA templates either directly after PCR or after cloning the PCR fragments into the pCR II vector. Ten of the mutant pRNA species were inactive in packaging of the vphi-29 genome. Mutation of base one at the 5' end did not affect the pRNA packaging activity. Mutation of the first two bases at the 5' end of the pRNA to noncomplementary bases in the predicted **RNA secondary structure** (U-1 C-2/A-117G-116 to G-1 G-2/A-117G-116) resulted in a pRNA with no detectable DNA-gp3 packaging activity assayed by either sucrose gradient sedimentation or agarose gel electrophoresis, and 10-5-fold **reduction** in activity was found when measured by plaque-forming units with a new highly sensitive assay system. Changing bases 11 6 and 11 7 so that they were complementary to the mutated bases, 1 and 2, from the previous mutant (G-1 G-2/A-117G-116 to G-1 G-2/C-11-7C-116) generated an **RNA** molecule with restored DNA packaging ability. Our results show that, although not essential for procapsid binding, both the 5' and 3' ends of the pRNA were proximate and crucial for vphi-29 DNA packaging.

1994

4/3,AB/120 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08852651 BIOSIS NO.: 199396004152
Structural studies of the enveloped dsRNA bacteriophage vphi-06 of Pseudomonas syringae by Raman spectroscopy: II. Nucleocapsid **structure** and thermostability of the virion, nucleocapsid and **polymerase** complex.
AUTHOR: Bamford Jaana K H; Bamford Dennis H; Li Tiansheng; Thomas George J Jr(a)
AUTHOR ADDRESS: (a)Div. Cell Biology Biophysics, Sch. Biological Sciences, Univ. Mo.-Kansas City, Kansas City, MO 6**USA
JOURNAL: Journal of Molecular Biology 230 (2):p473-482 1993
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Structures and thermostabilities of the double-stranded (ds) **RNA** bacteriophage vphi-6 and of its isolated nucleocapsid-**polymerase** complex (nucleocapsid core) and dsRNA components have been investigated by Raman spectroscopy. The spectra show that proteins of the vphi-6 virion are collectively deficient in beta-sheet **secondary structure**. In particular, the major protein (P8) of the outer spherical shell of the vphi-6 nucleocapsid exhibits a

secondary structure dominated largely by alpha-helix and irregular conformations. The absence of appreciable beta-**structure** in the P8 subunit suggests a tertiary conformation lacking the beta-barrel motif common to subunits of most other spherical viral capsids. In addition, the Raman spectra show that subunits of the dodecahedral nucleocapsid core are also predominantly alpha-helical. The results thus indicate a largely alpha-helical **secondary structure** for the major subunit (P1) of the vphi-6 nucleocapsid core, as well as for the P8 subunit of the outer spherical shell. Using Raman difference spectroscopy, we demonstrate that proteins of the nucleocapsid core (P1, P2, P4 and P7) interact extensively with the packaged vphi-6 **RNA** genome, and further, that conformational stability of the packaged **RNA** is **reduced** upon removal from the core. Also, we find that proteins of the vphi-6 nucleocapsid are significantly more thermostable than proteins of the viral membrane envelope, which are reported in the accompanying paper (Li et al., 1993). The present results suggest that both the architectural principles and modes of protein-**RNA** interaction of the vphi-6 virion differ fundamentally from those of icosahedral single-stranded DNA viruses. Both Raman and circular dichroism spectra indicate that the dsRNA genome of vphi-6 is an A-form **structure**. The Raman marker bands signify the presence only of C3'-endo/anti nucleoside conformers. The Raman signature of dsRNA, revealed in the spectrum of the vphi-6 genome, is discussed here as a model for assessing base-pairing and base-stacking interactions in other ribonucleoprotein assemblies.

1993

4/3,AB/121 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08753762 BIOSIS NO.: 199395043113
Fluorine-19 nuclear magnetic resonance as a probe of the solution **structure** of mutants of 5-fluorouracil-substituted Escherichia coli valine tRNA.
AUTHOR: Chu Wen-Chy; Feiz Vahid; Derrick Wesley B; Horowitz Jack(a)
AUTHOR ADDRESS: (a)Dep. Biochem. Biophysics, Mol. Cell. Dev. Biol. Program, Iowa State Univ., Ames, Iowa 50011
JOURNAL: Journal of Molecular Biology 227 (4):p1164-1172 1992
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In order to utilize 19F nuclear magnetic resonance (NMR) to probe the solution **structure** of Escherichia coli tRNA-Val labeled by incorporation of 5-fluorouracil, we have assigned its 19F spectrum. We describe here assignments made by examining the spectra of a series of tRNA-Val mutants with nucleotide substitutions for individual 5-fluorouracil residues. The result of base replacements on the **structure** and function of the tRNA are also characterized. Mutants were prepared by oligonucleotide-directed mutagenesis of a cloned tRNA-Val gene, and the tRNAs transcribed in vitro by bacteriophage T7 **RNA polymerase**. By identifying the missing peak in the 19F NMR spectrum of each tRNA variant we were able to assign resonances from fluorouracil residues in loop and stem regions of the tRNA. As a result of the assignment of FU33, FU34 and FU29, temperature-dependent spectral shifts could be attributed to changes in anticodon loop and stem conformation. Observation of a magnesium ion-dependent splitting of the resonance assigned to FU64 suggested that the T-arm of tRNA-Val can exist in two conformations in slow exchange on the NMR time scale. Replacement of most 5-fluorouracil residues in loops and stems had little effect on

the **structure** of tRNA-Val; few shifts in the 19F NMR spectrum of mutant tRNAs were noted. However, replacing the FU29 cntdot A41 base-pair in the anticodon stem with C29 cntdot G41 induced conformational changes in the anticodon loop as well as in the P-10 loop. Effects of nucleotide substitution on aminoacylation were determined by comparing the V-max and K-m values of tRNA-Val mutants with those of the wild-type tRNA. Nucleotide substitution at the 3' end of the anticodon (position 36) **reduced** the aminoacylation efficiency (V-max/K-m) of tRNA-Val by three orders of magnitude. Base replacement at the 5' end of anticodon (position 34) had only a small negative effect on the aminoacylation efficiency. Substitution of the FU29 cntdot A41 base-pair increased the K-m value 20-fold, while V-max remained almost unchanged. The FU4 cntdot A69 base-pair in the acceptor stem, could readily be replaced with little effect on the aminoacylation efficiency of E. coli tRNA-Val, indicating that this base-pair is not an identity element of the tRNA, as suggested by others.

1992

4/3,AB/122 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08091909 BIOSIS NO.: 000093101982
BIOCHEMICAL MECHANISMS OF RESISTANCE TO A NEW ANTITUMOUR NITROSOUREA DERIVATIVE CRC 680578
AUTHOR: GUDTSOVA K V; KUKUSHKINA G V; GORBACHEVA L B
AUTHOR ADDRESS: INST. CHEM. PHYS., ACAD. SCI. RUSS., MOSCOW, RUSS.
JOURNAL: BIOKHIMIYA 56 (8). 1991. 1509-1521. 1991
FULL JOURNAL NAME: Biokhimiya
CODEN: BIOHA
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: The biochemical mechanisms of resistance to CRC 680578, a new antitumor chloroethylnitrosourea .alpha.-amino acid derivative, were studied. Alterations in DNA, **RNA** and protein syntheses, SH-group content, drug efflux, activities of replicative and repair enzymes, such as ribonucleotide **reductase**, thymidine kinase, O6-alkylguanine-DNA-Alkyltransferase and DNA **polymerase** .alpha. and .beta. and damages of the DNA **secondary structure** were investigated in sensitive and resistant to CRC 680578 leukemia L1210 cells. It was found that the total SH-group number in drug-resistant cells was increased (about 1.3-fold in comparison with sensitive cells) which seems to be due to the mechanisms of drug resistance. CHC 680578 induced less pronounced inhibition and more rapid restoration of DNA and **RNA** synthesis in resistant cells. No differences between the ribonucleotide **reductase** and thymidine kinase activities were found either in intact cells of the both strains or after drug administration. The efficiency of repair of DNA chloroethyl adducts by O6-alkylguanine-DNA-alkyltransferase in leukemia cells of various sensitivity was found to be identical. The differences in enzyme activities in intact cells of the both strains were insignificant. It was supposed that factors other than changes in the level of O6-alkylguanine-DNA-alkyltransferase in leukemia cells may be responsible for the resistance to CRC 580578. The increase in the levels of DNA **polymerase** .alpha. and, especially, of DNA **polymerase** .beta., in sensitive (but not resistant) mouse leukemia cells 48 hours after drug administration is though to define the mechanism of resistance to the new antitumor agent CHC 680578.

1991

4/3,AB/123 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06775132 BIOSIS NO.: 000088084566
TRANSCRIPTION REGULATION IN-VITRO BY AN ESCHERICHIA-COLI PROMOTER CONTAINING A DNA CRUCIFORM IN THE MINUS 35 REGION
AUTHOR: HORWITZ M S Z
AUTHOR ADDRESS: THE JOSEPH GOTTSTEIN MEML. CANCER RES. LAB., DEP. PATHOL. SM-30, SCH. MED., UNIV. WASHINGTON, SEATTLE, WA 98195, USA.
JOURNAL: NUCLEIC ACIDS RES 17 (14). 1989. 5537-5546. 1989
FULL JOURNAL NAME: Nucleic Acids Research
CODEN: NARHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A promoter with the potential to adopt a 50 basepair (bp) cruciform spanning from -19 to -69 has been constructed in the plasmid pBR 322 tetracycline resistance gene (tet) by forming an inverted repeat from '-35' sequences. Compared to a control promoter, the sequence of this cruciform promoter differs only by a 22 bp insertion between -48 and -69, upstream from the usual location of promoter sequences. The cruciform is extruded in a supercoil-dependent manner, and transcription from this promoter in vitro by **RNA polymerase** decreases as the negative supercoil density of the plasmid DNA increases. In contrast, transcription from the control promoter increases with negative supercoiling. Thus, DNA **secondary structure** in the '-35' region can affect promoter-**polymerase** interaction. The tet promoter cruciform also influences expression of the pBR322 .beta.-lactamase gene (bla). This apparently results when extrusion of the cruciform **reduces** the superhelicity of the plasmid molecule to a level that is below the optimum for expression from the bla promoter, illustrating one mechanism for how DNA **secondary structure** may effect action-at-a-distance. Transcription from both promoters in vivo does not differ from controls, suggesting that this cruciform is not generated to a significant extent intracellularly, most probably as a result of the slow kinetics of extrusion.

1989

4/3,AB/124 (Item 23 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04249012 BIOSIS NO.: 000077075057
MUTATIONS OF THE BETA SUBUNIT OF **RNA POLYMERASE** ALTER BOTH TRANSCRIPTION PAUSING AND TRANSCRIPTION TERMINATION IN THE TRP OPERON LEADER REGION IN-VITRO
AUTHOR: FISHER R F; YANOFSKY C
AUTHOR ADDRESS: DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY, STANFORD, CALIFORNIA 94305.
JOURNAL: J BIOL CHEM 258 (13). 1983. 8146-8150. 1983
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: **RNA polymerase** was purified from rifampicin-resistant mutants of Escherichia coli which exhibit altered transcription termination at the trp operon attenuator in vivo. These mutant polymerases were used to investigate transcription pausing at the trp leader pause site and transcription termination at the trp attenuator.

The mutant polymerases examined in vitro mimic their in vivo termination responses; i.e., **RNA polymerase** isolated from a mutant which displays high transcriptional read-through of the trp operon in vivo allows greater transcriptional read-through in vitro, while **RNA polymerase** prepared from a mutant which has **reduced** read-through in vivo exhibits greater termination of transcription in vitro. The observed differences are not due to the presence of, or rsponse to, alternate **secondary** structures in the trp leader transcript since deletion of the DNA segment corresponding to some of these alternate structures does not affect termination efficiency. The mutant polymerases also have comparable effects on the kinetics of transcription pausing at the trp leader pause site; the termination-deficient **polymerase** exhibits diminished pausing while the termination-proficient **polymerase** displays enhanced pausing. This correlation may reflect **polymerase** recognition of similar features of **RNA secondary** structures in the pause and termination events. Since single mutational changes in **RNA polymerase** affect 2 activities, pausing and termination, a single site or region of the **polymerase** is probably involved in both events.

1983

```
s

Set      Items   Description
S1       10838   SECONDARY AND STRUCTURE AND RNA
S2        1615   S1 AND POLYMERASE
S3         163   S2 AND REDUC?
S4         124   RD (unique items)
S5           0   S4 AND ANALOG
? s s45 and t7
>>>"S45" does not exist
                0  S45
            11935  T7
      S6        0  S45 AND T7
? s s1 and t7
            10838  S1
            11935  T7
      S7      187  S1 AND T7
? s s7 not s4
              187  S7
              124  S4
      S8      169  S7 NOT S4
? s s8 not s3
              169  S8
              163  S3
      S9      158  S8 NOT S3
? s s9 and analog?
              158  S9
           587314  ANALOG?
     S10       10  S9 AND ANALOG?
? rd
...completed examining records
     S11        6  RD (unique items)
? t s11/3,ab/all

 11/3,AB/1      (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10441073   99436120   PMID: 10506170
     Bipartite   modular   structure   of   intrinsic,   RNA
 hairpin-independent termination signal for phage RNA polymerases.
  Kwon Y S; Kang C
  Department of Biological Sciences, Korea Advanced Institute of Science
and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea.
  Journal of biological chemistry (UNITED STATES)  Oct 8 1999,  274  (41)
 p29149-55,  ISSN 0021-9258   Journal Code: 2985121R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The phage SP6 RNA and T7 RNA polymerases, which are
closely related to each other, intrinsically stop at two signals in the
Escherichia coli rrnB terminator t1 through different mechanisms. The
downstream signal functioned without an RNA secondary
structure formation, in which the signal was still active when
separated from the upstream, hairpin-forming signal, and IMP incorporation
enhanced its efficiency. The sequence from -15 to -1 was essential for the
downstream, hairpin-independent termination (at -1). The results of SP6
transcription with heteroduplex templates and ribonucleotide analogs
suggested that the downstream signal consists of two functionally different
modules. The effects of iodo-CMP or IMP incorporation into RNA on
termination efficiency were not sensitive to incorporation at -9 and
upstream, but they were reactive to incorporation at -6 and -2, as
reflected by strong iodo-rC:dG and weak rI:dC base pairing. Thus, the
downstream module (from -8 approximately -6 to -1) appears to facilitate
```

WEST

Generate Collection | Print

Search Results - Record(s) 1 through 10 of 75 returned.

☐ 1. Document ID: US 20020198362 A1

L16: Entry 1 of 75 File: PGPB Dec 26, 2002

PGPUB-DOCUMENT-NUMBER: 20020198362
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020198362 A1

TITLE: Compositions and methods for the detection, diagnosis and therapy of hematological malignancies

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gaiger, Alexander	Seattle	WA	US	
Algate, Paul A.	Issaquah	WA	US	
Mannion, Jane	Seattle	WA	US	

US-CL-CURRENT: 530/350; 435/320.1, 435/325, 435/6, 435/69.1, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw Desc | Image

☐ 2. Document ID: US 20020197669 A1

L16: Entry 2 of 75 File: PGPB Dec 26, 2002

PGPUB-DOCUMENT-NUMBER: 20020197669
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020197669 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bangur, Chaitanya S.	Seattle	WA	US	
Fanger, Gary Richard	Mill Creek	WA	US	
Wang, Aijun	Issaquah	WA	US	
Wang, Tongtong	Medina	WA	US	
Switzer, Ann P.	Seattle	WA	US	
McNeill, Patricia D.	Federal Way	WA	US	
Clapper, Jonathan D.	Seattle	WA	US	

US-CL-CURRENT: 435/69.1; 435/183, 435/320.1, 435/325, 435/6, 536/23.2

Preparation and characterization of N-(1-pyrenyl)iodoacetamide-labeled Escherichia coli **RNA polymerase**.
Johnson R S; Bowers M; Eaton Q
Department of Biochemistry, East Carolina University School of Medicine, Greenville, North Carolina 27858.
Biochemistry (UNITED STATES) Jan 8 **1991**, 30 (1) p189-98, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
N-(1-Pyrenyl)iodoacetamide has been used to introduce fluorescent probes into Escherichia coli **RNA polymerase**. After an incubation time of 15 min, approximately 2 pyrene equiv was introduced per enzyme molecule. There was no further increase in modification after more extended periods of incubation. Neither calf thymus DNA nor nucleotides protected the holoenzyme from modification. Thus, the sites of modification do not appear to involve the binding sites for polynucleotides or the ribonucleoside triphosphates. From the isolation and analysis of the individual subunits, it was found that sigma contained approximately 1 pyrene equiv, beta contained 0.6, beta' contained 0.6, and alpha less than 0.1. Spectral and Stern-Volmer analyses indicate that the covalently attached pyrene molecules are in comparable apolar microenvironments. On the basis of CD analyses, the introduction of pyrene molecules into **RNA polymerase** alters its secondary structure. This alteration in secondary structure manifests itself by a reduction in overall enzymatic activity. Transcript analysis of the products obtained by using a linearized plasmid containing the A1 promoter and the Te terminator of bacteriophage **T7** indicates that the pyrenyl derivative is capable of producing full-length transcripts and that it has an efficiency of chain termination comparable to the native enzyme. Analysis of tau plots for the interaction of the pyrenyl derivative and the native enzyme, respectively, with the A1 promoter yielded comparable values for the isomerization constant in the conversion of the closed complex to an open one. Comparable values were also obtained for the association constant. The rate of chain elongation for the pyrenyl derivative, however, is approximately 54% of that observed for the native enzyme. Thus, the decrease in overall transcriptional activity observed with the pyrenyl derivative is not due to a decrease in the efficiency of initiation or premature termination, but rather to a decrease in the rate of chain elongation.

5/3,AB/105 (Item 105 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06798430 91120063 PMID: 2278831
The steroid-binding properties of recombinant glucocorticoid receptor: a putative role for heat shock protein hsp90.
Ohara-Nemoto Y; Stromstedt P E; Dahlman-Wright K; Nemoto T; Gustafsson J A; Carlstedt-Duke J
Department of Medical Nutrition, Karolinska Institutet, Huddinge University Hospital, Sweden.
Journal of steroid biochemistry and molecular biology (ENGLAND) Nov 30 **1990**, 37 (4) p481-90, ISSN 0960-0760 Journal Code: 9015483
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The steroid-binding domain of the human glucocorticoid receptor was expressed in Escherichia coli either as a fusion protein with protein A or under control of the **T7 RNA polymerase** promoter. The recombinant proteins were found to bind steroids with the normal specificity for a glucocorticoid receptor but with reduced affinity (Kd for triamcinolone acetonide approximately 70 nM). Glycerol gradient analysis of

the E. coli lystate containing the recombinant protein indicated no interaction between the glucocorticoid receptor fragment and heat shock proteins. However, synthesis of the corresponding fragments of glucocorticoid receptor in vitro using rabbit reticulocyte lystate resulted in the formation of proteins that bound triamcinolone acetonide with high affinity (Kd 2nM). Glycerol gradient analysis of these proteins, with and without molybdate, indicated that the in vitro synthesised receptor fragments formed complexes with hsp90 as previously shown for the full-length rat glucocorticoid receptor. Radiosequence analysis of the recombinant steroid-binding domain expressed in E. coli and affinity labelled with dexamethasone mesylate identified binding of the steroid to Cys-638 predominantly. However, all cysteine residues within the steroid-binding domain were affinity labelled to a certain degree indicating that the recombinant protein has a structure similar to the native receptor but more open and accessible.

5/3,AB/106 (Item 106 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06786449 91099349 PMID: 2269294
Eukaryotic DNA replication. Enzymes and proteins acting at the fork.
Thommes P; Hubscher U
Department of Pharmacology and Biochemistry, University Zurich-Irchel, Switzerland.
European journal of biochemistry / FEBS (GERMANY) Dec 27 **1990**, 194 (3) p699-712, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A complex network of interacting proteins and enzymes is required for DNA replication. Much of our present understanding is derived from studies of the bacterium Escherichia coli and its bacteriophages T4 and **T7**. These results served as a guideline for the search and the purification of **analogous** proteins in eukaryotes. model systems for replication, such as the simian virus 40 DNA, lead the way. Generally, DNA replication follows a multistep enzymatic pathway. Separation of the double-helical DNA is performed by DNA helicases. Synthesis of the two daughter strands is conducted by two different DNA polymerases: the leading strand is replicated continuously by DNA **polymerase** delta and the lagging strand discontinuously in small pieces by DNA **polymerase** alpha. The latter is complexed to DNA primase, an enzyme in charge of frequent **RNA** primer syntheses on the lagging strand. Both DNA polymerases require several auxiliary proteins. They appear to make the DNA polymerases processive and to coordinate their functional tasks at the replication fork. 3'----5'-exonuclease, mostly part of the DNA **polymerase** delta polypeptide, can perform proof-reading by excising incorrectly base-paired nucleotides. The short DNA pieces of the lagging strand, called Okazaki fragments, are processed to a long DNA chain by the combined action of RNase H and 5'----3'-exonuclease, removing the **RNA** primers, DNA **polymerase** alpha or beta, filling the gap, and DNA ligase, sealing DNA pieces by phosphodiester bond formation. Torsional stress during DNA replication is released by DNA topoisomerases. In contrast to prokaryotes, DNA replication in eukaryotes not only has to create two identical daughter strands but also must conserve higher-order structures like chromatin.

5/3,AB/107 (Item 107 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06785367 91097779 PMID: 2268428
A simple assay for determining the capping efficiencies of **RNA** polymerases used for in vitro transcription.

Theus S A; Liarakos C D
Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock 72205.
BioTechniques (UNITED STATES) Nov **1990**, 9 (5) p610-2, 614-5, ISSN 0736-6205 Journal Code: 8306785
Contract/Grant No.: GM41257; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have developed an assay that uses phenyl boronate agarose column chromatography to measure the capping efficiencies of **RNA** polymerases used for in vitro transcription of cloned cDNAs. Capped 32P-labeled ovalbumin mRNAs were synthesized by in vitro run-off transcription with SP6 or **T7 RNA polymerase** in the presence of cap **analogs** and digested to completion with T1 and T2 RNase. The resulting 3'-nucleoside monophosphates (NMPs) and cap structures were separated by chromatography on phenyl boronate agarose, and the ratio of radioactivity between the two was used to estimate the extent of transcript capping.

5/3,AB/108 (Item 108 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06781543 91091980 PMID: 1702368
Role of DNA **polymerase** 3'----5' exonuclease activity in the bypass of aminofluorene lesions in DNA.
Strauss B S; Wang J
Department of Molecular Genetics and Cell Biology, University of Chicago, IL 60637.
Carcinogenesis (UNITED STATES) Dec **1990**, 11 (12) p2103-9, ISSN 0143-3334 Journal Code: 8008055
Contract/Grant No.: CA 32436; CA; NCI; GM07816; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
N-(Deoxyguanosin-8-yl)-2-(acetylamino)fluorene (AAF-G) adducts in the DNA of bacteriophage M13 can be converted to N-(deoxyguanosin-8-yl)-2-aminofluorene (AF-G) adducts in situ by treatment with 1.0 M NaOH for 45 min at room temperature. The conversion is accompanied by a dramatic increase in the transfection activity of the samples which is correlated with the measured deacetylation of the acetylaminofluorene adduct. The pair of substrates (AAF-G/AF-G) with adducts at identical places in the DNA has been used to study bypass synthesis catalyzed by **T7** DNA **polymerase**, an altered **T7** DNA **polymerase** from which the 3'----5' exonuclease has been genetically removed by an 84 nucleotide deletion (Sequenase 2), T4 DNA **polymerase** and Escherichia coli DNA **polymerase** I. All polymerases appear blocked at acetylaminofluorene lesions. Sequenase 2 is apparently able to add nucleotides opposite the acetylaminofluorene lesion but is unable to catalyze further elongation. **T7** DNA **polymerase**, including thioredoxin and with an active 3'----5' exonuclease, is unable to bypass aminofluorene adducts, whereas Sequenase 2 bypasses the lesions readily. The data support the view that the elongation step is rate limiting in synthesis past lesions and that low 3'----5' exonuclease activity allows the priming nucleotide opposite the altered template site to remain in position long enough for elongation past particular adducts.

5/3,AB/109 (Item 109 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06669186 90366625 PMID: 2393673
The study of nucleoside triphosphate-binding center of DNA-dependent

RNA-polymerase of phage **T7** using GTP **analogs**]
Issledovanie nukleozidtrifosfatsviazyvaiushchego tsentra DNK- zavisimoi RNK-polimerazy faga **T7** s pomoshch'iu **analogov** GTP.
Akbarov A Kh; Tunitskaia V L; Baranova L A; Khropov Iu V; Krasil'nikova M M; Kochetkov S N
Biokhimii a (Moscow, Russia) (USSR) May **1990**, 55 (5) p829-35, ISSN 0320-9725 Journal Code: 0372667
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
The NTP binding site of bacteriophage **T7** DNA-dependent **RNA polymerase** was studied using GTP **analogs**. For four **analogs** the irreversible inhibition was demonstrated. The kinetic parameters for competitive (Ki) and irreversible (KI and k3) inhibition were determined. One of the **analogs**, 5'[2-hydroxy(4-iodoacetamido)benzoyl]guanosine, was shown to inactivate the enzyme rapidly due to the modification of SH-groups. Some suggestions on the structure of the **RNA polymerase** active site have been made.

5/3,AB/110 (Item 110 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06639539 90336647 PMID: 1696203
Inactivation of bacteriophage **T7** DNA-dependent **RNA polymerase** by 5'-p-fluorosulfonylbenzoyladenosine. Identification of the modification site and the effect of the modification on enzyme action.
Tunitskaya V L; Akbarov A Kh; Luchin S V; Memelova L V; Rechinsky V O; Kochetkov S N
V. A. Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Moscow.
European journal of biochemistry / FEBS (GERMANY, WEST) Jul 20 **1990**, 191 (1) p99-103, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Bacteriophage **T7 RNA polymerase** was covalently modified by 5'-[4-fluorosulfonyl)benzoyl]adenosine (4-FSO2BzAdo). The modified enzyme lacks the ability to catalyze **RNA** synthesis from the phi 10 promoter of bacteriophage **T7** ; both promoter and GTP binding being markedly decreased. The mild hydrolysis of the ester bond of 4-FSO2BzAdo within the covalent enzyme-inhibitor complex restores the **RNA** synthesis at a lower rate. Sequence studies show that Lys172 is the target of modification by 4-FSO2BzAdo. This residue, which is situated in the polypeptide region connecting two domains of **RNA polymerase**, was shown to be the primary site of the limited proteolysis occurring in vivo [Ikeda, R. A. & Richardson, C. C. (1987) J. Biol. Chem. 262, 3790-3799]. We propose that Lys172 is located outside the active site. Once this residue has reacted with 4-FSO2BzAdo, the nucleoside moiety of the **analog** is fixed in the NTP-binding site of the active centre and prevents binding of the substrates. Here, Lys172 per se is not important for the activity but serves as an 'anchor' for binding of the inhibitor.

5/3,AB/111 (Item 111 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06639054 90335966 PMID: 2143105
The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal.
Hamm J; Darzynkiewicz E; Tahara S M; Mattaj I W
European Molecular Biology Laboratory, Heidelberg, Federal Republic of

Germany.
Cell (UNITED STATES) Aug 10 **1990**, 62 (3) p569-77, ISSN 0092-8674 Journal Code: 0413066
Contract/Grant No.: 2507-RR 05356; RR; NCRR; GM 38512; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The ability of series of U1 snRNAs and U6 snRNAs to migrate into the nucleus of Xenopus oocytes after injection into the cytoplasm was analyzed. The U snRNAs were made either by injecting U snRNA genes into the nucleus of oocytes or, synthetically, by **T7 RNA polymerase**, incorporating a variety of cap structures. The results indicate that nuclear targeting of U1 snRNA requires both a trimethylguanosine cap structure and binding of at least one common U snRNP protein. Using synthetic U6 snRNAs, it is further demonstrated that the trimethylguanosine cap structure can act in nuclear targeting in the absence of the common U snRNP proteins. These results imply that U snRNP nuclear targeting signals are of a modular nature.

5/3,AB/112 (Item 112 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06620239 90320144 PMID: 2371774
Infectious in vitro transcripts from a plum pox potyvirus cDNA clone.
Riechmann J L; Lain S; Garcia J A
Centro de Biologia Molecular, CSIC-UAM, Universidad Autonoma de Madrid, Spain.
Virology (UNITED STATES) Aug **1990**, 177 (2) p710-6, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A full-length cDNA clone of the 9786 nt plum pox virus (PPV) **RNA** genome has been cloned downstream from a phage **T7 RNA polymerase** promoter. The RNAs synthesized by in vitro run-off transcription in the presence of the 5' cap **analog** m7GpppG were infectious in Nicotiana clevelandii plants. No infectivity was detected when the transcriptions were carried out in the absence of the cap **analog** . Inoculations of the local lesion host Chenopodium foetidum indicated that the infectivity of the synthetic transcripts was about 1% of that of the native viral **RNA**. An extra G present at the 5' terminus of the transcripts was lost during their replication in plants, and the typical length distribution of the poly(A) tails was recovered. The viral **RNA** recovered from transcript-infected plants had approximately the same specific infectivity as native viral **RNA** . A G/A sequence heterogeneity found between different cDNA subgenomic clones was used to demonstrate that the infections were caused by the in vitro transcripts and were not the result of contamination.

5/3,AB/113 (Item 113 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06616261 90314960 PMID: 1695096
T7 RNA polymerase: use of limited proteolysis for the study of enzyme's interaction with substrates and inhibitors.
Tunitskaya V L; Akbarov A Kh; Kochetkov S N
V.A. Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Moscow.
Biochemistry international (AUSTRALIA) **1990**, 20 (6) p1033-40, ISSN 0158-5231 Journal Code: 8100311

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510.
Biochemistry (UNITED STATES) Apr 4 **1989**, 28 (7) p2760-2,
ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM10902; GM; NIGMS; GM21919-13; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The study of transcription kinetics by **T7 RNA polymerase** is facilitated by the small size of its promoter, allowing the use of synthetic oligonucleotide templates with carefully defined sequences. We have previously used this approach to measure Michaelis-Menten steady-state kinetics for production of the five-base runoff transcript GGACU. In particular, Km for the interaction between enzyme and template under saturating levels of all four nucleotide triphosphates was shown to be approximately 0.02 microM. We now show that the corresponding Km and Vmax for initiation on a similar template coding for the runoff transcript GACU are the same as for the earlier study (Km = 0.02 microM; kcat = 40-50 min-1). This new template allows the measurement Km for association of the initial nucleotide GTP with enzyme or with the enzyme-DNA complex. The results show that KGTPm (0.60 mM) is somewhat higher than earlier approximations of Km for addition of elongating GTP during the later phase of processive elongation. As expected, the (initiating) Km for the GTP **analogue** ITP (KITPm) is increased (by about 2-fold), presumably as a result of weakened Watson-Crick base pairing. However, comparison of Km values for the GTP **analogues** GMP and guanosine shows little effect on substitution of the 5'-triphosphate by monophosphate or by a hydroxyl, respectively. This result suggests that a single active site has been evolutionarily adapted to accept from the 5' end of a waiting nucleotide both a 5'-triphosphate at initiation and a 5'-monophosphate ester (**RNA**) during elongation.(ABSTRACT TRUNCATED AT 250 WORDS)

5/3,AB/122 (Item 122 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06104772 89207489 PMID: 2853971
Binding interactions between yeast tRNA ligase and a precursor transfer ribonucleic acid containing two photoreactive uridine **analogues**.
Tanner N K; Hanna M M; Abelson J
Department of Biology, California Institute of Technology, Pasadena 91125.
Biochemistry (UNITED STATES) Nov 29 **1988**, 27 (24) p8852-61,
ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: 1 F32 GM11823; GM; NIGMS; GM 32637; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Yeast tRNA ligase, from Saccharomyces cerevisiae, is one of the protein components that is involved in the splicing reaction of intron-containing yeast precursor tRNAs. It is an unusual protein because it has three distinct catalytic activities. It functions as a polynucleotide kinase, as a cyclic phosphodiesterase, and as an **RNA** ligase. We have studied the binding interactions between ligase and precursor tRNAs containing two photoreactive uridine **analogues** , 4-thiouridine and 5-bromouridine. When irradiated with long ultraviolet light, **RNA** containing these **analogues** can form specific covalent bonds with associated proteins. In this paper, we show that 4-thiouridine triphosphate and 5-bromouridine triphosphate were readily incorporated into a precursor tRNA(Phe) that was synthesized, in vitro, with bacteriophage **T7 RNA polymerase**. The **analogue** -containing precursor tRNAs were

hydrolysis and the resulting 2' and 3' nucleoside monophosphates separated by reverse-phase HPLC. The amount of 32P transferred to each monophosphate was indicative of the frequency of their incorporation into the transcript. Transcripts synthesized in the presence of equimolar concentrations of Bio-4-UTP and UTP resulted in 70 out of the 125 possible UTP sites occupied by Bio-4-UMP. This study agrees with kinetic data in suggesting that **T7 RNA polymerase** does not significantly discriminate between the natural and the biotinylated nucleotide. Therefore, the number of biotinylated residues that are incorporated into a transcript can be controlled by varying the ratio of Bio-4-UTP to UTP in the transcription reaction. We have shown that as few as 10 Bio-4-UMP residues per 486 nucleotide transcript still results in greater than 90% binding efficiency on a streptavidin/biotin-cellulose affinity column.

5/3,AB/103 (Item 103 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06803400 91105138 PMID: 1703019
Effects of 2-chloro-2'-deoxyadenosine 5'-triphosphate on DNA synthesis in vitro by purified bacterial and viral DNA polymerases.
Hentosh P; McCastlain J C; Blakley R L
Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.
Biochemistry (UNITED STATES) Jan 15 **1991**, 30 (2) p547-54, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: P30 CA 21765; CA; NCI; RO1 CA 39242; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
2-Chloro-2'-deoxyadenosine 5'-triphosphate (CldATP) was compared with dATP as a substrate for DNA synthesis by bacterial and viral DNA polymerases in vitro. Lengths of chain extension and DNA synthesis pause sites were determined by comparison with products generated by dideoxynucleotide sequencing methods on the same end-labeled primer/template duplex after high-resolution polyacrylamide gel electrophoresis. Reverse transcriptase (RT) from human immunodeficiency virus (HIV-1) and avian myeloblastosis virus (AMV) incorporated CldATP efficiently. DNA strand elongation continued past most chloroadenine (ClA) insertion sites but resulted in shorter chains than when dATP was inserted. Phage T4 DNA **polymerase** incorporated CldATP least efficiently; Klenow fragment of Escherichia coli DNA **polymerase** I and modified **T7** DNA **polymerase** (Sequenase) showed intermediate ability to utilize the **analogue**. Incorporation of several consecutive ClA residues into the replicating strand dramatically reduced the ability of Sequenase, Klenow fragment, and T4 DNA polymerases to continue strand elongation. In the absence of the corresponding normal deoxyribonucleoside triphosphate during DNA synthesis, ClA was frequently misincorporated as thymine, cytosine, or guanine by both AMV RT and HIV-1 RT but rarely, if at all, by Klenow fragment, Sequenase, and T4 DNA **polymerase**. Except T4, for most DNA polymerases, CldATP at 10-20-fold molar excess over dATP was not a strong competitive inhibitor of dATP, as judged by the amount of strand extension and **polymerase** pause sites during DNA synthetic reactions. Our results indicate that the degree of strand extension in the presence of CldATP, the number and location of **polymerase** pause sites, and the amount of misincorporation of the **analogue** are both **polymerase**- and sequence-dependent.

5/3,AB/104 (Item 104 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06803342 91105080 PMID: 1988020

Biokhimii a (Moscow, Russia) (RUSSIA) Jan **1993**, 58 (1) p43-9, ISSN 0320-9725 Journal Code: 0372667
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed

Interactions of the bacteriophage **T7** DNA-dependent **RNA polymerase** with three GTP **analogs** have been studied. All of the three **analogs** tested contained substituted naphthalenesulphamide groups and were shown to be under appropriate conditions irreversible covalent inhibitors of the enzyme, the modified enzyme possessing fluorescent properties. One of these **analogs** contained the reactive 2-bromoethyl phosphonate group and was shown to cause the loss of the enzyme affinity for polynucleotide templates. The other two modifiers which contained the azide reactive group did not alter the enzyme-template affinity, the polynucleotide binding leading to a notable increase of the enzyme fluorescence intensity. The latter two modifiers are supposed to be convenient for fluorescent labelling of the active site of **RNA polymerase** for enzyme-template binding studies.

5/3,AB/79 (Item 79 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07671144 93194890 PMID: 8449951

Effects of DNA lesions on transcription elongation by **T7 RNA polymerase**.

Chen Y H; Bogenhagen D F

Department of Pharmacological Sciences, State University of New York, Stony Brook 11794-8651.

Journal of biological chemistry (UNITED STATES) Mar 15 **1993**, 268 (8) p5849-55, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: ES04068; ES; NIEHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

T7 phage **RNA polymerase** was used to transcribe a series of DNA templates bearing any of several precisely localized lesions. Lesions were positioned downstream of the **T7** promoter on either strand of the DNA template to investigate the effects of these lesions on elongation of transcription. The following four types of DNA modifications were studied: 1) 3-hydroxy-2-hydroxymethyltetrahydrofuran (tetrahydrofuran), a synthetic apurinic/apyrimidinic site; 2) 8-oxoguanine (8-oxodG), an oxidized derivative of guanine; 3) N-acetyl-2-aminofluorene (AAF) modified guanine; 4) 2-aminofluorene (AF) modified guanine. None of these lesions blocked transcription elongation when they were located on the non-template strand. Lesions on the template strand blocked elongation with varied efficiency. The series of AAF-dG, AF-dG, and tetrahydrofuran lesions showed a progressively decreasing ability to block elongation, while 8-oxo-dG caused little, if any, premature termination. **T7 RNA polymerase** was able to read through all of the lesions with sufficient efficiency to permit chain termination sequencing using the read-through products as templates. AAF-dG and AF-dG adducts did not induce detectable misreading. Adenine and, more rarely, cytosine were incorporated opposite 8-oxo-dG, as observed for translesional synthesis by DNA polymerases. Adenine was most commonly inserted opposite the non-instructional abasic site **analogue**, although a minor fraction of guanine was incorporated.

5/3,AB/80 (Item 80 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07598341 93126085 PMID: 1282702
Recognition of the high affinity binding site in rev-response element **RNA** by the human immunodeficiency virus type-1 rev protein.
Iwai S; Pritchard C; Mann D A; Karn J; Gait M J
MRC Laboratory of Molecular Biology, Cambridge, UK.
Nucleic acids research (ENGLAND) Dec 25 **1992**, 20 (24) p6465-72
, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The Human Immunodeficiency Virus type-1 rev protein binds with high affinity to a bubble structure located within the rev-response element (RRE) **RNA** in stemloop II. After this initial interaction, additional rev molecules bind to the RRE **RNA** in an ordered assembly process which requires a functional bubble structure, since mutations in the bubble sequence that reduce rev affinity block multiple complex formation. We have used synthetic chemistry to characterize the interaction between rev protein and its high affinity binding site. A minimal synthetic duplex **RNA** (RBC6) carrying the bubble and 12 flanking base pairs is able to bind rev with 1 to 1 stoichiometry and with high affinity. When the bubble structure is inserted into synthetic **RNA** molecules carrying longer stretches of flanking double-stranded **RNA** , rev forms additional complexes resembling the multimers observed with the RRE **RNA**. The ability of rev to bind to RBC6 **analogues** containing functional group modifications on base and sugar moieties of nucleoside residues was also examined. The results provide strong evidence that the bubble structure contains specific configurations of non-Watson--Crick G:G and G:A base pairs and suggest that high affinity recognition of RRE **RNA** by rev requires hydrogen bonding to functional groups in the major groove of a distorted **RNA** structure.

5/3,AB/81 (Item 81 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07568878 93094202 PMID: 1281153
Beta-L-thymidine 5'-triphosphate **analogs** as DNA **polymerase** substrates.
Van Draanen N A; Tucker S C; Boyd F L; Trotter B W; Reardon J E
Division of Experimental Therapy, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.
Journal of biological chemistry (UNITED STATES) Dec 15 **1992**, 267 (35) p25019-24, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
beta-L-3'-Deoxythymidine 5'-triphosphate (L-ddTTP) and beta-L-3'-deoxy-2',3'-didehydrothymidine 5'-triphosphate (L-d4TTP) were substrates for human immunodeficiency virus reverse transcriptase, Escherichia coli DNA **polymerase** I (Klenow), and Sequenase (modified **T7** DNA **polymerase**). The beta-D- and beta-L-enantiomers of 5-methyluridine 5'-triphosphate (rTTP) were inhibitors but not substrates of reverse transcriptase. The steady-state Km values for L-ddTTP and L-d4TTP, with all three enzymes, were 12-70-fold larger than the Km values for the corresponding D-enantiomers. The Km value of reverse transcriptase for L-ddTTP was 50-fold larger than that for D-ddTTP because the Kd for L-ddTTP was 5-fold larger than that for D-ddTTP, and the first-order rate constant for incorporation of L-ddTMP into the template-primer was 10% that of the D-enantiomer. The D- and L-enantiomers had kcat values with reverse transcriptase and Sequenase that were similar to kcat for the natural substrate, thymidine 5'-triphosphate (dTTP). Thus, the rate determining step appeared to be dissociation of the enzyme-chain-terminated

template-primer complex. In contrast, kcat values for the L-enantiomers with Klenow were only 0.1% that of dTTP, and the kcat values for the D-enantiomers were 15% the kcat for dTTP. The reduced kcat values were due to a change in rate determining step from dissociation of the Klenow-chain-terminated template-primer complex to an earlier step in the reaction mechanism, presumably catalysis. Thus, these DNA polymerases did not stereospecifically recognize D-nucleoside 5'-triphosphate **analogs** as substrates.

5/3,AB/82 (Item 82 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07527994 93054909 PMID: 1429835
GTP-binding mutants of rab1 and rab2 are potent inhibitors of vesicular transport from the endoplasmic reticulum to the Golgi complex.
Tisdale E J; Bourne J R; Khosravi-Far R; Der C J; Balch W E
Department of Cell Biology, Scripps Research Institute, La Jolla, California 92037.
Journal of cell biology (UNITED STATES) Nov **1992**, 119 (4) p749-61, ISSN 0021-9525 Journal Code: 0375356
Contract/Grant No.: CA27489; CA; NCI; GM33301; GM; NIGMS; GM42336; GM; NIGMS; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have examined the role of ras-related rab proteins in transport from the ER to the Golgi complex in vivo using a vaccinia recombinant **T7 RNA polymerase** virus to express site-directed rab mutants. These mutations are within highly conserved domains involved in guanine nucleotide binding and hydrolysis found in ras and all members of the ras superfamily. Substitutions in the GTP-binding domains of rab1a and rab1b (equivalent to the ras 17N and 116I mutants) resulted in proteins which were potent trans dominant inhibitors of vesicular stomatitis virus glycoprotein (VSV-G protein) transport between the ER and cis Golgi complex. Immunofluorescence analysis indicated that expression of rab1b121I prevented delivery of VSV-G protein to the Golgi stack, which resulted in VSV-G protein accumulation in pre-Golgi punctate structures. Mutants in guanine nucleotide exchange or hydrolysis of the rab2 protein were also strong trans dominant transport inhibitors. **Analogous** mutations in rab3a, rab5, rab6, and H-ras did not inhibit processing of VSV-G to the complex, sialic acid containing form diagnostic of transport to the trans Golgi compartment. We suggest that at least three members of the rab family (rab1a, rab1b, and rab2) use GTP hydrolysis to regulate components of the transport machinery involved in vesicle traffic between early compartments of the secretory pathway.

5/3,AB/83 (Item 83 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07508467 93033116 PMID: 1413505
Transcription dependence of DNA packaging of bacteriophages T3 and **T7**.
Hashimoto C; Fujisawa H
Department of Botany, Faculty of Science, Kyoto University, Japan.
Virology (UNITED STATES) Nov **1992**, 191 (1) p246-50, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
T3 and **T7** phages package homologous DNA more efficiently than

heterologous DNA and recombinant plasmids carrying DNA sequences necessary for DNA packaging (pac sequence). The pac sequence contains a promoter for phage **RNA polymerase** and transcription from the promoter is necessary for DNA packaging. T3 and **T7 RNA** polymerases are stringently specific for their own promoters. To examine the relationship between DNA packaging and transcription, we constructed a cleared in vitro system for packaging T3 or **T7** DNA containing an ammonium sulfate fractionate of a high-speed supernatant of phage-infected cells. In the system, DNA packaging required GTP and was inhibited by the 3'-deoxy **analog** of GTP, ATP, or CTP. The DNA packaging activity paralleled the transcriptional activity, assayed by incorporation of [32P]UTP into acid-insoluble material. In the system, homologous DNA was packaged more efficiently than heterologous DNA, but heterologous DNA was packaged as efficiently as homologous DNA by the addition of heterologous phage **RNA polymerase** , demonstrating that the transcriptional specificity determines the DNA packaging specificity of T3 and **T7**.

5/3,AB/84 (Item 84 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07476869 93003118 PMID: 1390741
2'-Fluoro- and 2'-amino-2'-deoxynucleoside 5'-triphosphates as substrates for **T7 RNA polymerase**.
Aurup H; Williams D M; Eckstein F
Max-Planck-Institut fur experimentelle Medizin, Gottingen, FRG.
Biochemistry (UNITED STATES) Oct 13 **1992**, 31 (40) p9636-41, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
2'-Fluoro- and 2'-amino-2'-deoxynucleoside 5'-triphosphates have been investigated as substrates for **T7 RNA polymerase** . Michaelis-Menten kinetic parameters are reported for the incorporation of 2'-fluoro-2'-deoxyuridine, 2'-fluoro-2'-deoxycytidine, and 2'-amino-2'-deoxyuridine into runoff transcripts. The 2'-amino derivative of uridine is a better substrate than the 2'-fluoro derivative. Gel electrophoretic analysis shows that full-length transcripts with a length of 2500 nucleotides can be obtained with the **analogues** , although a considerable amount of shorter fragments accompanies the full-length product. In keeping with the kinetic analysis, the 2'-aminouridine triphosphate gives a cleaner product than the 2'-fluoro **analogue**. Transcription of two tRNA genes shows that such shorter templates can be transcribed to full-length products essentially without premature termination with any of the **analogues**.

5/3,AB/85 (Item 85 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07467354 92408009 PMID: 1527853
Internal entry of ribosomes on a tricistronic mRNA encoded by infectious bronchitis virus.
Liu D X; Inglis S C
Department of Pathology, University of Cambridge, United Kingdom.
Journal of virology (UNITED STATES) Oct **1992**, 66 (10) p6143-54, ISSN 0022-538X Journal Code: 0113724
Erratum in J Virol 1992 Nov;66(11) 6840
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
mRNA3 specified by the coronavirus infectious bronchitis virus appears to

be functionally tricistronic, having the capacity to encode three small proteins (3a, 3b, and 3c) from separate open reading frames (ORFs). The mechanism by which this can occur was investigated through in vitro translation studies using synthetic mRNAs containing the 3a, 3b, and 3c ORFs, and the results suggest that translation of the most distal of the three ORFs, that for 3c, is mediated by an unconventional, cap-independent mechanism involving internal initiation. This conclusion is based on several observations. A synthetic mRNA whose peculiar 5' end structure prevents translation of the 5'-proximal ORFs (3a and 3b) directs the synthesis of 3c normally. Translation of 3c, unlike that of 3a and 3b, was insensitive to the presence of the 5' cap **analog** 7-methyl-GTP, and it was unaffected by alteration of the sequence contexts for initiation on the 3a and 3b ORFs. Finally, an mRNA in which the 3a/b/c infectious bronchitis virus coding region was placed downstream of the influenza A virus nucleocapsid protein gene directed the efficient synthesis of 3c as well as nucleocapsid protein, whereas initiation at 3a and 3b could not be detected. Expression of the 3c ORF from this mRNA, however, was abolished when the 3a and 3b coding region was deleted, indicating that 3c initiation is dependent on upstream sequence elements which together may serve as a ribosomal internal entry site similar to those described for picornaviruses.

5/3,AB/86 (Item 86 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07414831 92347348 PMID: 1379176
Three widely separated positions in the 16S **RNA** lie in or close to the ribosomal decoding region; a site-directed cross-linking study with mRNA **analogues**.
Dontsova O; Dokudovskaya S; Kopylov A; Bogdanov A; Rinke-Appel J; Junke N ; Brimacombe R
Department of Chemistry, Moscow State University, Russia.
EMBO journal (ENGLAND) Aug **1992**, 11 (8) p3105-16, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Synthetic mRNA **analogues** were prepared by **T7** transcription, each containing several thio-uridine residues at selected positions. After binding to the ribosome in the presence of cognate tRNA, the thio-U residues were activated by UV irradiation and the resulting sites of cross-linking to 16S **RNA** analysed. Three distinct cross-links were consistently observed: (i) from position '+6' of the mRNA (the 3'-base of the A-site codon) to base 1052 of 16S **RNA**; (ii) from position '+7' of the mRNA to base 1395; and (iii) from '+11' to base 532. Individual yields of the cross-links were strongly dependent on the particular mRNA sequence in each case. The '+11/532' and '+6/1052' cross-links were always entirely tRNA-dependent, whereas the '+7/1395' cross-link was observed at lower intensity in the absence of tRNA. In the presence of a second (A-site bound) tRNA the +6/1052 cross-link was markedly reduced. A cross-link to the 1050 region was again observed when a message carrying a thio-U at position '+9' was translocated on the ribosome so as to bring the thio-U to position +6. Taken together, the data are incompatible with some current models both for the three-dimensional arrangement of 16S **RNA** and for the orientation of the tRNA-mRNA complex in the ribosome.

5/3,AB/87 (Item 87 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07413292 92345329 PMID: 1379079
Identification of the Escherichia coli 30S ribosomal subunit protein

neighboring mRNA during initiation of translation.
Dontsova O A; Rosen K V; Bogdanova S L; Skripkin E A; Kopylov A M; Bogdanov A A
Department of Chemistry, Moscow State University, Russia.
Biochimie (FRANCE) Apr **1992**, 74 (4) p363-71, ISSN 0300-9084 Journal Code: 1264604
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To identify the proteins of the 30S ribosomal subunit of E coli that neighbor mRNA in the ternary initiation complex (mRNA*30S subunit*tRNA(fMet), we used an affinity cross-linking approach in which photoactivated groups were attached to different positions along the mRNA chain. A series of mini-genes originating from the 5'-end region of the cro gene of lambda bacteriophage were constructed as templates for mini-mRNA synthesis. Two strategies were used to introduce photo-reactive agents into the message. According to the first, two transcripts were isolated from E coli and chemically derivatized at their 5'-ends with a photoinducible diaziril group. One of these messages allowed for localization of the 5'-end of the Shine-Dalgarno sequence while the other one allowed for labeling of the ribosome at the 5'-end side of the initiation AUG codon in the P site. According to the second approach, 5-azidouridine (5N3U) was randomly incorporated into mRNA transcripts during a **T7 RNA polymerase** catalyzed reaction by using a mixture of 5N3UTP and UTP. A message that had U residues at either -4, -3, -1, +2 and +14, +19, +20 positions was used (A from cro AUG is +1). Whereas cross-links with the 5N3U transcripts were essentially 'zero-length', the 5'-derivatized transcripts were covalently attached to ribosomal components about 14 A from the 5'-end. We found that proteins S1, S7, S5, S3 and S4 compose, or were close to, the ribosomal mRNA-binding site.(ABSTRACT TRUNCATED AT 250 WORDS)

5/3,AB/88 (Item 88 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07396214 92329494 PMID: 1627602
Mapping of the active site of **T7 RNA polymerase** with 8-azidoATP.
Knoll D A; Woody R W; Woody A Y
Department of Biochemistry, Colorado State University, Fort Collins 80523.
Biochimica et biophysica acta (NETHERLANDS) Jun 24 **1992**, 1121 (3) p252-60, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: GM-23697; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The photoaffinity **analog** of ATP, 8-azidoATP, labels **T7 RNA polymerase**. Photoincorporation exhibits saturation behavior and is protected against by the substrate ATP. 8-AzidoATP is a competitive inhibitor of ATP incorporation with Ki approximately 40 microM. The photolabeled **T7 RNA polymerase** , following cyanogen bromide digestion, was analyzed by phenylboronate agarose column chromatography followed by reverse-phase high pressure liquid chromatography. Sequencing of the peptides labeled with radioactive photoprobe allowed the identification of three peptides, P314-M362 (I), L550-M666 (II), and F751-M861 (III). These peptides are in the proximity of the photoprobe 8-azidoATP and, therefore, expected to contain functionally significant residues and define an active site domain. These peptides (I and II) contain residues previously implicated in **T7 RNA polymerase** activity or show homology to active site regions of the

Klenow fragment of DNA **polymerase** I (II and III).

5/3,AB/89 (Item 89 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07387887 92319642 PMID: 1620609
Joints formed by RecA protein from oligonucleotides and duplex DNA block initiation and elongation of transcription.
Golub E I; Ward D C; Radding C M
Department of Genetics, Yale University School of Medicine, New Haven, CT 06510.
Nucleic acids research (ENGLAND) Jun 25 **1992**, 20 (12) p3121-5, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM 40633; GM; NIGMS; HG 00338; HG; NHGRI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the presence of the non-hydrolyzable **analog** of ATP, ATP gamma S, RecA protein can polymerize on an oligodeoxy-ribonucleotide to form a stable oligonucleoprotein filament that can find its homologous sequence in double-stranded DNA. The homologous joint formed by the oligonucleotide and duplex DNA is stable only if RecA protein is not removed. Such a nucleoprotein joint, covering a part or all of the promoter region of T3 or **T7** phage **RNA polymerase**, blocked transcription directed by those polymerases. The same kind of joint, located downstream of the **RNA polymerase** promoter, also inhibited elongation of transcription and caused accumulation of truncated transcripts. These observations suggest that RecA protein can be used to shut off transcription from any promoter of known sequence.

5/3,AB/90 (Item 90 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07338908 92271227 PMID: 1589782
Site-specific modification of pre-mRNA: the 2'-hydroxyl groups at the splice sites.
Moore M J; Sharp P A
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139.
Science (UNITED STATES) May 15 **1992**, 256 (5059) p992-7, ISSN 0036-8075 Journal Code: 0404511
Contract/Grant No.: P30-CA14051; CA; NCI; RO1 GM34277; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A simple and efficient method for synthesizing long, site-specifically modified **RNA** molecules was developed whereby segments of **RNA** were joined with the use of bacteriophage T4 DNA ligase. A single hydrogen or O-methyl group was substituted for the 2'-hydroxyl group at either splice site of a nuclear pre-messenger **RNA** substrate. Splicing of the modified pre-messenger **RNA** 's in vitro revealed that, although a 2'-hydroxyl is not absolutely required at either splice site, the 2'-hydroxyl at the 3' splice site is important for the second step of splicing. These results are compared to previous studies of **analogous** 2'-hydroxyl groups in the self-splicing Tetrahymena group I intron.

5/3,AB/91 (Item 91 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07221661 92144548 PMID: 1737000
Binding of protein synthesis initiation factor 4E to oligoribonucleotides: effects of cap accessibility and secondary structure.
Carberry S E; Friedland D E; Rhoads R E; Goss D J
Department of Chemistry, Hunter College of the City University of New York 10021-5024.
Biochemistry (UNITED STATES) Feb 11 **1992**, 31 (5) p1427-32, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM 20818; GM; NIGMS; RR-0307; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The binding of rabbit globin mRNA to the 25-kDa cap binding protein eIF-4E from human erythrocytes was found to be 5.3-fold stronger than the binding of the cap **analogue** m7GpppG to eIF-4E [Gross et al. (1990) Biochemistry 29, 5008-5012]. In order to investigate whether this effect is due to the longer sequence of nucleotides in globin mRNA or to other features such as cap accessibility or secondary structure, oligoribonucleotide **analogues** of rabbit alpha-globin mRNA were synthesized by **T7 RNA polymerase** from a synthetic oligodeoxynucleotide template in the presence of m7GpppG; these oligoribonucleotide **analogues** possess varying degrees of cap accessibility and secondary structure. Equilibrium association constants for the interaction of these oligoribonucleotides and purified human erythrocyte eIF-4E were obtained from direct fluorescence titration experiments. The data indicate that while the presence of the m7G cap is required for efficient recognition by eIF-4E, the cap need not be completely sterically accessible, since other structural features within the mRNA also influence binding.

5/3,AB/92 (Item 92 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07176980 92112043 PMID: 1662654
High-efficiency protein synthesis from **T7 RNA polymerase** transcripts in 3T3 fibroblasts.
Deng H; Wang C; Acsadi G; Wolff J A
Department of Pediatrics, Waisman Center, University of Wisconsin, Madison 53705.
Gene (NETHERLANDS) Dec 30 **1991**, 109 (2) p193-201, ISSN 0378-1119 Journal Code: 7706761
Contract/Grant No.: HD00669-05; HD; NICHD; HD03352; HD; NICHD
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
After NIH3T3 cells constitutively expressing **T7 RNA polymerase** were transfected (+ Ca.phosphate) with a circular DNA containing the firefly luciferase(Luc)-encoding gene (luc) 3' to the encephalomyocarditis (EMC) virus 5'-untranslated sequence and **T7** promoter, Luc protein comprising approx. 20% of total cellular protein was obtained. After similar transfection of an **analogous** construct containing the lacZ gene into the same cell line, at least 50% of the cells produced beta-galactosidase. Fibroblasts lipofected with uncapped **RNA** transcripts containing EMC sequence expressed the reporter genes as efficiently as capped transcripts. A novel approach was used to generate **RNA** transcripts containing poly(A) at its very 3' end. **RNA** from a luc vector with a poly(A) sequence at the very 3' end produced 20-fold more Luc than the **RNA** from the same vector with an additional 3' nonpoly(A) sequence. These results suggest that this **T7 RNA polymerase** expression system will be useful for the efficient production of proteins in mammalian cells.

5/3,AB/93 (Item 93 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07175524 92109706 PMID: 1764057
Gene expression from multicopy **T7** promoter vectors proceeds at single copy rates in the absence of **T7 RNA polymerase**.
Somerville R L; Shieh T L; Hagewood B; Cui J S
Department of Biochemistry, Purdue University West Lafayette, IN 47907.
Biochemical and biophysical research communications (UNITED STATES) Dec 31 **1991**, 181 (3) p1056-62, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Three different genes (trpR+, tyrR+ and phi (trpR-lacZ)) were inserted into pET3a, a multicopy transcription-translation vector designed by Rosenberg et al. (1) for the **T7 RNA polymerase**-driven overexpression of proteins in Escherichia coli. Gene orientation was in the anticlockwise ("silent") direction. Gene expression in the absence of **T7 RNA polymerase** was evaluated either directly using lacZ reporter systems or indirectly by observing the susceptibility of plasmid-bearing tester strains to inhibition by an aromatic amino acid **analog** . The production of repressor proteins and of a Trp repressor-LacZ chimera was readily detected, at levels comparable to those of haploid trpR+ or tyrR+ E. coli strains. Such **T7** vector constructs thus have two especially useful properties: first, they provide a means for the high-level production of various proteins in E. coli; second, they offer a technically advantageous point of departure for structure-function studies of genes whose overexpression from multicopy plasmids would normally be cytotoxic.

5/3,AB/94 (Item 94 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07114612 92046053 PMID: 1942045
Studies on the interaction of **T7 RNA polymerase** with a DNA template containing a site-specifically placed psoralen cross-link. II. Stability and some properties of elongation complexes.
Sastry S S; Hearst J E
Department of Chemistry, University of California, Berkeley.
Journal of molecular biology (ENGLAND) Oct 20 **1991**, 221 (4) p1111-25, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM 41911; GM; NIGMS; NIEHS 07075-11; EH; NCEH
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We constructed a 66 base-pair DNA template capable of supporting transcription by **T7 RNA polymerase**. This template had a psoralen cross-link downstream from a **T7** promoter such that a 36 (+1) nucleotide transcript was synthesized at the time the **T7 polymerase** came to a stop at the cross-link. The stability of elongation complexes formed on this template, and the effect of different factors that are known to affect **polymerase**-DNA interactions was investigated by non-denaturing gel electrophoresis and gel filtration chromatography. We found that an elongation complex could lose its **RNA** component but the **T7 polymerase** still remained attached to the DNA template for extended periods of time (at least up to 18 h). This type of an elongation complex, bereft of its nascent **RNA** transcript, is called a quasi-elongation complex. DNase I footprinting within gel slices indicated that the **polymerase** molecules were

arrested at the psoralen cross-link on the DNA template in the quasi-elongation complexes. The quasi-elongation complexes were found to be extremely stable in 0.5 M-NaCl and in 0.2 M-NaCl plus 60 mM-MgCl2, and could withstand temperatures up to 42 degrees C. The quasi-elongation complexes were destabilized by heparin and excess calf thymus DNA. Excess tRNA caused only a minimal degree of disruption. Non-promoter-containing plasmid DNAs did not have a destabilizing effect on the quasi-elongation complexes. Interestingly, it was observed that in a **T7** ternary transcriptional complex arrested by a psoralen cross-link, the nascent **RNA** transcript could be stabilized from release by the presence in trans of a plasmid DNA bearing a **T7** promoter and a **T7** terminator. Such a stabilization against **RNA** release was not observed with plasmid DNAs containing either only a promoter or a terminator. The elongation complexes were stable during gel filtration through Sephacryl S-300 HR. However, it was found that 30% to 45% of the labeled **RNA** was retained during gel filtration as **RNA** that was apparently free from ternary complexes.

5/3,AB/95 (Item 95 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07114611 92046052 PMID: 1942044

Studies on the interaction of **T7 RNA polymerase** with a DNA template containing a site-specifically placed psoralen cross-link. I. Characterization of elongation complexes.

Sastry S S; Hearst J E
Department of Chemistry, University of California, Berkeley.

Journal of molecular biology (ENGLAND) Oct 20 **1991**, 221 (4) p1091-110, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM 41911; GM; NIGMS; NIEHS 07075-11; EH; NCEH
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A 66 base-pair (bp) DNA template carrying a site-specifically placed psoralen cross-link downstream from a phage **T7** promoter was constructed. This template can support transcription by **T7 RNA polymerase** . Transcription was blocked specifically at the psoralen cross-link. We studied the characteristics of elongation complexes, formed in this manner, by enzymatic and chemical footprinting and by a nitrocellulose filter-binding assay. The DNase I footprint of the elongation complex was quantified on a residue by residue basis. It was found that **T7 RNA polymerase** made the strongest contacts in the central region of the footprint whereas the leading and lagging edges of the **polymerase** were weakly bound to the DNA. Reducing the NaCl concentration in the transcription reaction resulted in the visualization of two **T7 RNA polymerase** molecules bound to the same template. A leading **polymerase** molecule, arrested at the psoralen cross-link, showed a much smaller DNase I footprint than a lagging **polymerase** molecule that was bound upstream. This upstream **polymerase** molecule occupied approximately one-half of the promoter region and therefore did not achieve complete promoter clearance. These experiments suggest that complete promoter clearance is required for a gross conformational change in the **polymerase** , consisting of a contraction in the size of its footprint to occur. DNase I footprinting also revealed that an elongation complex arrested at a psoralen cross-link undergoes several subtle changes in structure in a time-dependent manner and therefore can be considered to be in a state of dynamic flux. Methylation protection showed that some G residues in the top (non-coding) strand are protected against attack by dimethylsulfate, whereas the G residues on the bottom (coding) strand appear not to be protected from reaction with dimethylsulfate. We probed the transcribing complexes for single-stranded regions with **T7** gene 3 endonuclease. From the pattern

of sensitivity to **T7** gene 3 endonuclease on the template strand, we conclude that the **RNA**-DNA hybrid in the elongation complex is about 7 bp. A nitrocellulose filter-binding assay showed that the elongation complex, consisting of a 36 (+1) nucleotide **RNA**, the 66 bp DNA template and the **T7 RNA polymerase** was stable for at least 30 minutes at high salt concentrations. Heparin caused the quantitative release of 36 (+1) **RNA** nucleotides within 30 seconds, but the DNA was not simultaneously released from the elongation complex under these conditions.

5/3,AB/96 (Item 96 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07098134 92031550 PMID: 1932016
RNA folding during transcription by **T7 RNA polymerase** analyzed using the self-cleaving transcript assay.
Tyagarajan K; Monforte J A; Hearst J E
Department of Chemistry, University of California, Berkeley.
Biochemistry (UNITED STATES) Nov 12 **1991**, 30 (45) p10920-4, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM41911; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have used a self-cleaving **RNA** molecule (a "hammerhead") to study the length-dependent folding of **RNA** produced during transcription by **T7 RNA polymerase**. Transcript elongation is arrested at defined positions using chain-terminating ribonucleoside triphosphate **analogues** , 3'-deoxynucleoside triphosphates. When the nascent transcript attains the minimum length required for the "hammerhead" domain of the transcript to fully emerge from the ternary complex, the "hammerhead" structure forms and self-cleaves, producing a truncated product. The experiment yields an **RNA** sequencing ladder which terminates at the length at which cleavage becomes possible; the sequencing ladder is compared to that generated by using a noncleaving control template. We have shown that 13 nucleotides past the cleavage point must be synthesized before the transcript can self-cleave in the ternary complex whereas **RNA** freed from the complex by heating can cleave with only 3 or more nucleotides present beyond the cleavage site. The results indicate that the **RNA** in **T7 RNA polymerase** is not free of steric interactions in the ternary complex and not available for structure formation until it is at least 10 bases away from the site of polymerization. The results suggest that the maximum possible length of the **RNA** -DNA hybrid in the ternary complexes is 10. The relevance of the results in comparisons with other **RNA** polymerases, especially Escherichia coli **RNA polymerase**, is discussed.

5/3,AB/97 (Item 97 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07098056 92031472 PMID: 1718418
A C-nucleotide base pair: methylpseudouridine-directed incorporation of formycin triphosphate into **RNA** catalyzed by **T7 RNA polymerase**.
Piccirilli J A; Moroney S E; Benner S A
Laboratory for Organic Chemistry, Swiss Federal Institute of Technology, Zurich.
Biochemistry (UNITED STATES) Oct 22 **1991**, 30 (42) p10350-6, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
With templates containing 2'-deoxy-1-methylpseudouridine (dm psi), **T7 RNA polymerase** catalyzes the incorporation of either adenosine triphosphate (ATP) or formycin triphosphate (FTP) into a growing chain of **RNA** with the same efficiency as with templates containing thymidine (dT). In each case, the overall rate of synthesis of full-length products containing formycin is about one-tenth of the rate of synthesis of **analogous** products containing adenosine. Analysis of the products of abortive initiation shows that incorporation of FMP into the growing oligonucleotide by **T7 RNA polymerase** is more likely to lead to premature termination of transcription than is incorporation of AMP. Nevertheless, the results demonstrate that **T7 RNA polymerase** tolerates the formation of a C-nucleotide transcription complex in which the nucleoside bases on both the template and the incoming nucleotide are joined to the ribose by a carbon-carbon bond. This result increases the prospects for further expanding the genetic alphabet via incorporation of new base pairs with novel hydrogen-bonding schemes (Piccirilli et al., 1990).

5/3,AB/98 (Item 98 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07076218 92011516 PMID: 1655731
Spin-labeled nucleotide substrates for DNA-dependent **RNA polymerase** from Escherichia coli.
Tyagi S C
Department of Biochemistry and Cell Biology, State University of New York, Stony Brook 11794-5215.
Journal of biological chemistry (UNITED STATES) Sep 25 **1991**, 266 (27) p17936-40, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
New spin-labeled **analogs** of nucleoside triphosphates, 8-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)adenosine 5'-triphosphate ((8-AmTEMPO)ATP) and 5-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)uridine 5'-triphosphate ((5-AmTEMPO)UTP), with the probe 4-amino(2,2,6,6-tetramethy lpiperidine-N-oxyl) (4-AmTEMPO) attached to C-8 of ATP and C-5 of UTP via a secondary amine bond, were synthesized in 50 and 40% yield, respectively. These **analogs** showed a single spot by thin layer chromatographic analysis. The absorption spectra of (8-Am-TEMPO)ATP and (5-AmTEMPO)UTP exhibit maxima at 310 and 265 nm, respectively; their X-band EPR spectra have a typical three-line pattern with lines at 3,221, 3,239, and 3,257 Gauss. The intensity ratios for mid to high field lines of the EPR derivative lines were found to be 1.03 +/- 0.02, 1.08 +/- 0.04, and 1.15 +/- 0.07 for 4-AmTEMPO, (8-AmTEMPO)ATP, and (5-AmTEMPO)UTP, respectively. The immobilization of 4-AmTEMPO bound to C-8 of ATP or bound to C-5 of UTP was observed to be 5 and 11%, respectively, as compared with free 4-AmTEMPO. The initial velocity (s-1) of [3H]UMP incorporation into **RNA** in the presence of [3H]UTP, CTP, GTP, and (8-AmTEMPO)ATP or ATP was measured. The percent incorporation of (8-AmTEMPO)ATP into **RNA** product by Escherichia coli **RNA polymerase** using various DNA templates is 68, 66, and 61% for pAR1435 (plasmid containing A1 promoter from **T7** DNA), calf thymus DNA, and poly(dA-dT) respectively, as compared with ATP incorporation. The **polymerase**-catalyzed reaction of (8-AmTEMPO)ATP with (3'-OCH3)UTP yielded 5'-triphosphate delta-amino(2,2,6,6-tetramethylpiperidine-N-oxyl)adenylyl (3'-5')3'-methoxy uridine in the presence of poly(dA-dT). The structure of this spin-labeled dinucleotide was identified by paper chromatographic analysis of the products of phosphodiesterase digestion. These **analogs** also can be used for the study by EPR spectroscopy of the dynamics of gene

transcription catalyzed by **RNA** polymerases or of other nucleotide-utilizing enzymes.

5/3,AB/99 (Item 99 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06980763 91293095 PMID: 1712292
The path of mRNA through the Escherichia coli ribosome; site-directed cross-linking of mRNA **analogues** carrying a photo-reactive label at various points 3' to the decoding site.
Rinke-Appel J; Junke N; Stade K; Brimacombe R
Max-Planck-Institut fur Molekulare Genetik, Abteilung Wittmann, Berlin, FRG.
EMBO journal (ENGLAND) Aug **1991**, 10 (8) p2195-202, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
mRNA **analogues** approximately 40 bases long were prepared by **T7** transcription from synthetic DNA templates. Each message contained the sequence ACC-GCG (coding for threonine and alanine, respectively), together with a single thio-U residue located at a variable position on the 3'-side of these coding triplets. The thio-U residue was either substituted with 4-azidophenacyl bromide to introduce a photo-reactive group, or was left unsubstituted for direct UV cross-linking. After binding to Escherichia coli 70S ribosomes in the presence of tRNA-Thr or tRNA-Ala, the thio-U residue or azidophenyl group was photo-activated and the products of cross-linking (which was exclusively to the 30S subunit) were analysed. Immunological analysis of the cross-linked proteins showed that S5 and S3, together with S1, were the targets of cross-linking at positions close to the decoding site, with the cross-linking to S3 and S1 persisting at positions further away. Analysis of the 16S **RNA** showed cross-links to the region of bases 1390-1400 in all cases, but in one instance (with the reactive nucleotide 11 bases from the decoding site) simultaneous cross-linking was observed to the latter region and to position 532; these two **RNA** regions are far apart in current three-dimensional models of the 30S subunit.

5/3,AB/100 (Item 100 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06938053 91244994 PMID: 1645371
Comparison of biotinylated DNA and **RNA** probes for rapid detection of varicella-zoster virus genome by in situ hybridization.
Forghani B; Yu G J; Hurst J W
Division of Laboratories, California State Department of Health Services, Berkeley 94704.
Journal of clinical microbiology (UNITED STATES) Mar **1991**, 29 (3) p583-91, ISSN 0095-1137 Journal Code: 7505564
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We describe a general method for the production of nonisotopic DNA and **RNA** probes for the detection of the varicella-zoster virus (VZV) genome by in situ hybridization. VZV DNA was extracted from purified viral nucleocapsids, cleaved with restriction enzyme (RE) BamHI, and cloned into plasmid pBR322 by the standard vector insert procedure. We cloned over 85% of the VZV genome and obtained 18 recombinants. Plasmids containing the B, F, G, H, and J fragments of VZV DNA were labeled by the nick translation method with biotin-11-dUTP as the dTTP **analog**. Additionally, the B

fragment was cleaved with RE AvaI, subcloned into the plasmid pGEM-4 transcription vector, and subsequently linearized with REs PstI and EcoRI. **RNA** was transcribed with **T7** or SP6 **polymerase**, with a substitution of allylamine-UTP as the UTP **analog**, and labeled with epsilon-caproylamidobiotin-N-hydroxysuccinimide ester. The DNA and **RNA** probes were used under full-stringency conditions for in situ hybridization with alkaline phosphatase as the detector and 5-bromo-4-chloro-3-indolyl phosphate-Nitro Blue Tetrazolium as the substrate. When tested under comparable conditions, the **RNA** probe was slightly more sensitive than was the DNA probe: both probes showed homology only with VZV-infected cells and clinical tissues and not with the other herpesviruses. Probes prepared from variable regions of the genome (fragments F and J) performed as well as did those from conserved regions (fragments B. G. and H). Biotinylated probes have distinct advantages over isotopic probes and retain their full potency for more than 2 years when stored properly.

5/3,AB/101 (Item 101 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06854433 91153326 PMID: 1847871
Lys631 residue in the active site of the bacteriophage **T7 RNA polymerase**. Affinity labeling and site-directed mutagenesis.
Maksimova T G; Mustayev A A; Zaychikov E F; Lyakhov D L; Tunitskaya V L; Akbarov A Kh; Luchin S V; Rechinsky V O; Chernov B K; Kochetkov S N
Limnological Institute, Siberian Division of the USSR Academy of Sciences, Irkutsk.
European journal of biochemistry / FEBS (GERMANY) Feb 14 **1991**, 195 (3) p841-7, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A highly selective affinity labeling of **T7 RNA polymerase** with the o-formylphenyl ester of GMP and [alpha-32P]UTP was carried out. The site of the labeling was located using limited cleavages with hydroxylamine, bromine, N-chlorosuccinimide and cyanogene bromide and was identified as the Lys631 residue. Site-directed mutagenesis using synthetic oligonucleotides was used to substitute Lys631 by a Gly, Leu or Arg residue. Kinetic studies of the purified mutant enzymes showed alterations of their polymerizing activity. For the Lys----Gly mutant enzyme, anomalous template binding was observed.

5/3,AB/102 (Item 102 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06818557 91135938 PMID: 1704688
Direct quantitation of biotin-labeled nucleotide **analogs** in **RNA** transcripts.
Fenn B J; Herman T M
Department of Biochemistry, Medical College of Wisconsin, Milwaukee 53226.
Analytical biochemistry (UNITED STATES) Oct **1990**, 190 (1) p78-83, ISSN 0003-2697 Journal Code: 0370535
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have developed a method to determine directly the number of biotinylated (Bio) nucleotide **analogs** incorporated into **RNA** transcripts. Transcripts synthesized in vitro in the presence of [alpha 32-P]CTP and varying concentrations of Bio-4-UTP were subjected to alkaline

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The specific limited trypsinolysis of bacteriophage T7 **RNA polymerase** (T7RP) was performed in the presence of various components of the **polymerase** reaction and some GTP-**analogs**--irreversible inhibitors of the enzyme. The differences in the rate and sites of proteolysis were observed. Basing on the data obtained the role of the N-terminal domain of the T7RP in the interaction with promoter-containing template is proposed.

5/3,AB/114 (Item 114 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06437014 90119995 PMID: 2481987
Detection of DNA targets with biotinylated and fluoresceinated **RNA** probes. Effects of the extent of derivitization on detection sensitivity.
Folsom V; Hunkeler M J; Haces A; Harding J D
Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Maryland 20877.
Analytical biochemistry (UNITED STATES) Nov 1 **1989**, 182 (2) p309-14, ISSN 0003-2697 Journal Code: 0370535
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The substituted nucleotide aminohexyl-ATP (AH-ATP) was used for synthesis of **RNA** probes from a plasmid template using the **T7** phage promoter. Following synthesis, **RNA** probes were modified by reaction with N-hydroxysuccinimide (NHS) esters of biotin or fluorescein. Nearest-neighbor analysis was used to quantitate both the incorporation of the substituted nucleotide into **RNA** and the subsequent modification of the incorporated nucleotide by the NHS esters. The results indicate that AH-ATP is efficiently incorporated into **RNA** and that modification of the amine group is also efficient. The **T7 polymerase** shows a bias for ATP over AH-ATP and truncated transcripts are produced if 100% AH-ATP is used for synthesis. However, the use of 50% AH-ATP in the synthesis reaction yields full-length **RNA** probes that contain on average one amine-labeled nucleotide every 12 bases. This **RNA** is readily modified by the respective NHS esters to obtain one biotin group per 15-18 total **RNA** bases or one fluorescein group per 25-35 bases. Probes modified with biotin or fluorescein were used to detect picogram levels of target DNA in a dot blot hybridization format.

5/3,AB/115 (Item 115 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06418153 90114132 PMID: 2608036
Affinity modification of DNA-dependent **RNA-polymerase** from phage **T7** with 5'-p-fluorosulfonylbenzoyl adenosine: the effect of modification on the interaction with substrates]
Affinnaia modifikatsiia DNK-zavisimoi RNK-polimerazy faga **T7** 5'-p-ftorsul'fonilbenzoiladenozinom: vliianie modifikatsii na vzaimodeistvie s substratami.
Tunitskaia V L; Luchin S V; Memelova L V; Liakhov D L; Rechinskii V O; Kochetkov S N
Molekuliarnaia biologiia (USSR) Sep-Oct **1989**, 23 (5) p1273-8, ISSN 0026-8984 Journal Code: 0105454
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM

Record type: Completed
T7 RNA polymerase , covalently modified with 5'-p-fluorosulfonylbenzoyl adenosine, looses the ability of binding the promoter (pGEM-2 plasmid) and poly(dC) template as well as the initiating nucleoside triphosphate (GTP). However the enzyme retains the unspecific binding with DNA fragments of considerable length.

5/3,AB/116 (Item 116 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06308369 90001242 PMID: 2675976
Requirements for efficient in vitro transcription and translation: a study using yeast invertase as a probe.
Roitsch T; Lehle L
Lehrstuhl fur Zellbiologie und Pflanzenphysiologie, Universitat Regensburg, F.R.G.
Biochimica et biophysica acta (NETHERLANDS) Sep 21 **1989**, 1009 (1) p19-26, ISSN 0006-3002 Journal Code: 0217513
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Factors for efficient synthesis of mRNA in vitro and its subsequent translation in cell free lysates from reticulocyte and wheat germ were studied using yeast invertase as a probe. Among various transcription systems tested, containing either SP6, T5, **T7** or a bacterial synthetic consensus promoter, the **T7** system was superior both from a quantitative and qualitative point of view. Transcription with SP6 **polymerase** , but not with the other enzymes, resulted in premature transcript termination, which is ascribed to a sensitivity of the SP6 **polymerase** towards a hairpin loop structure in the invertase coding region. In-frame fusion of the critical DNA sequence to a different gene promoted premature transcription termination of the resulting chimeric template, which in its original form is transcribed correctly. Transcripts with additional sequences 5' upstream of the natural translation start revealed a diminished protein synthesis presumably due to the presence of out of frame ATG codons. In contrast, no influence on translation was found when additional sequences at the 3' end were present or when the stop codon was missing. Capping of transcripts was essential for translation in wheat germ lysates, whereas protein synthesis in reticulocytes was only reduced in the absence of a cap. The influence of polyadenylation on translation was studied using transcripts with engineered poly(A) tracts of different size. Increasing poly(A) chain length abolished translation in vitro in both translation systems. Inhibition was poly(A)-specific and is discussed as interference of the poly(A) sequences with a crucial component(s) of the protein synthesis machinery.

5/3,AB/117 (Item 117 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06290434 89377826 PMID: 2776753
Biochemical and biophysical analysis of pseudoknot-containing **RNA** fragments. Melting studies and NMR spectroscopy.
van Belkum A; Wiersema P J; Joordens J; Pleij C; Hilbers C W; Bosch L
Department of Biochemistry, Gorlaeus Laboratories, Leiden, The Netherlands.
European journal of biochemistry / FEBS (GERMANY, WEST) Aug 15 **1989**, 183 (3) p591-601, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Three overlapping **RNA** fragments containing the pseudoknot, as found in the tRNA-like structure of turnip yellow mosaic virus (TYMV) **RNA**, have been isolated and purified. Site-directed cleavage of TYMV **RNA** by RNase H, followed by ammonium sulphate precipitation and ion-exchange HPLC, yielded a pure preparation of a 3'-terminal, 112-nucleotide TYMV **RNA** fragment. Transcription of TYMV cDNA by **T7 RNA polymerase** , resulted in the isolation of an 88-nucleotide fragment. Finally, a 44-nucleotide fragment containing the TYMV **RNA** pseudoknot and strongly resembling the aminoacyl acceptor arm of the viral **RNA** was also synthesised using **T7 RNA polymerase**. The three fragments were isolated in milligram amounts and used for biochemical structure mapping, ultraviolet melting studies and NMR spectroscopy. Chemical modification with diethyl pyrocarbonate and sodium bisulphite and enzymatic digestion with RNase T1 confirmed the presence of the pseudoknot in the 44-nucleotide fragment. Also the **analogue** of the T-stem and T-loop of the tRNA-like structure of TYMV **RNA** was found. The results of modification at various temperatures in Mg2+-containing buffers were in general agreement with optical melting studies. Ultraviolet melting analysis of the longer fragments revealed their greater complexity and the results appear similar to those obtained for some tRNA species. To obtain direct biophysical evidence for base-pairing and stacking interactions in the pseudoknot, NMR studies were initiated. The first proton-NMR spectra ever obtained for plant viral **RNA** fragments are presented. NMR spectra were recorded at various buffer conditions and at various temperatures. The spectra for the 112-nucleotide and 88-nucleotide fragment are too complicated to be solved at present. In the case of the 44-nucleotide fragment, however, the imino proton resonances are well separated and this system turns out to be most promising for structural studies.

5/3,AB/118 (Item 118 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06280145 89364829 PMID: 2671676
Kinetics of inhibition by 8-oxy-GTP and 8-bromo-GTP of Escherichia coli **RNA-polymerase** synthesis of pppApU dinucleotide on the promotor a1 of phage T7deltaD111 DNA in a limited set of substrates]
Kinetika ingibirovaniia 8-oksi-GTP i 8-brom-GTP sinteza dinukleotida pppApU RNK-polimerazoi Escherichia coli na promotore A1 DNK faga T7deltaD111 pri ogranichennom nabore substratov.
Kuriavyi V V; Usacheva A M; Bruskov V I
Molekuliarnaia biologiia (USSR) May-Jun **1989**, 23 (3) p822-9,
ISSN 0026-8984 Journal Code: 0105454
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
Detailed analysis of the kinetics of inhibition of E. coli **RNA-polymerase** -catalyzed synthesis of dinucleotide pppApU by 8-oxy-GTP and 8-Br-GTP on promoter A1 of the bacteriophage **T7** delta D111 with an incomplete set of substrates was carried out. In accordance with the mathematical models obtained, we calculated quantitative parameters of binding of these nucleotide **analogs** to the centers whose geometry is suitable for incorporation of ATP and UTP. 8-oxy-GTP and 8-Br-GTP compete with ATP for the binding center (their steady-state dissociation constant ratios are 2.1 and 2.4, respectively, whereas the constant for ATP is 0.3 mM) but, unlike ATP, they are not incorporated into the product. 8-oxy-GTP competes also with UTP (its steady-state dissociation constant ratio is 21.6, the constant for UTP is 0.03 mM). 8-Br-GTP does not interact with the binding center of UTP.

,AB/119 (Item 119 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

06261298 89347990 PMID: 2763456
Infectious barley stripe mosaic virus **RNA** transcribed in vitro from full-length genomic cDNA clones.
Petty I T; Hunter B G; Wei N; Jackson A O
Department of Plant Pathology, University of California, Berkeley 94720.
Virology (UNITED STATES) Aug **1989**, 171 (2) p342-9, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Full-length genomic cDNA clones of the Type and ND18 strains of barley stripe mosaic virus (BSMV) were transcribed in vitro using **T7 RNA polymerase**. The combination of RNAs alpha, beta, and gamma synthesized in the presence of 5' cap **analogs** was infectious after inoculation onto barley plants, conclusively demonstrating the tripartite nature of the BSMV genome. Transcripts synthesized in the absence of cap **analogs** were not infectious. A gamma-specific subgenomic **RNA** which is normally present in BSMV virions was not required to establish a systemic infection. In vitro transcripts of variant cDNA clones which were isolated from the ND18 strain, containing either a simple nucleotide substitution or a 372-nucleotide duplication similar to one found in the genome of the Type strain, were also found to be biologically active. Two dicotyledonous hosts which have a differential response to infection with the Type and ND18 strains of BSMV were identified and these phenotypes were shown to be faithfully reproduced by inoculation with in vitro transcripts derived from the appropriate full-length cDNA clones.

5/3,AB/120 (Item 120 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06257067 89342440 PMID: 2760923
Nucleotide sequence and complementation studies of the gene 10 region of bacteriophage T3.
Condreay J P; Wright S E; Molineux I J
Department of Microbiology, University of Texas, Austin 78712.
Journal of molecular biology (ENGLAND) Jun 5 **1989**, 207 (3) p555-61, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM32095; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The nucleotide sequence of bacteriophage T3 gene 10 and surrounding regulatory elements has been determined and compared to the **analogous** region of bacteriophage **T7**. T3 genes 9, 10 and 11 have been shown to complement **T7** mutants. The DNA sequences of T3 and **T7** gene 10A are homologous, as are the amino acid sequences of the respective products. The translational shift to the -1 frame is predicted to occur at the same position in gene 10 of T3 and **T7** , though different nucleotide sequences are probably responsible. The resulting gp10B products have completely different C termini.

5/3,AB/121 (Item 121 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06219423 89302852 PMID: 2663058
T7 RNA polymerase does not interact with the 5'-phosphate of the initiating nucleotide.
Martin C T; Coleman J E

authentic substrates for the two splicing enzymes that were tested (endonuclease and ligase), and they formed specific covalent bonds with ligase when they were irradiated with long-wavelength ultraviolet light. We have determined the position of three major cross-links and one minor cross-link on precursor tRNA(Phe) that were located within the intron and near the 3' splice site. On the basis of these data, we present a model for the in vivo splicing reaction of yeast precursor tRNAs.

5/3,AB/123 (Item 123 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05993284 89083491 PMID: 3060847
Interaction of psoralen-derivatized oligodeoxyribonucleoside methylphosphonates with synthetic DNA containing a promoter for **T7 RNA polymerase**.
Lee B L; Blake K R; Miller P S
Division of Biophysics, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205.
Nucleic acids research (ENGLAND) Nov 25 **1988**, 16 (22) p10681-97, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: CA 42762; CA; NCI; GM 31927; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The interaction of 4'-N(2-aminoethyl)aminomethyl-4,5',8-trimethylpsoralen -modified oligonucleoside methylphosphonates with synthetic ds-DNA containing a **T7 RNA polymerase** promoter was studied. The oligomers effectively crosslinked with either coding or noncoding ss-DNA when irradiated at 365 nm, but not with ds-DNA. The extent of the crosslinking reaction, which was complete within 16 min: (a) reached its maximum at an oligomer concentration of 3 microM; (b) remained constant below the Tm of the duplex and then rapidly decreased; and (c) appeared to depend upon the sequence surrounding the psoralen crosslinking site. An oligomer crosslinked to the template strand inhibited transcription by **T7 RNA polymerase** whereas an oligomer crosslinked to the non-template strand had only a small inhibitory effect. Oligomers did not crosslink to ds-DNA undergoing transcription nor did they inhibit the transcription reaction.

5/3,AB/124 (Item 124 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05805786 88227847 PMID: 2836367
Sequence of the dnaB gene of Salmonella typhimurium.
Wong A; Kean L; Maurer R
Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.
Journal of bacteriology (UNITED STATES) Jun **1988**, 170 (6) p2668-75, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: AI-19942; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The dnaB gene of Escherichia coli encodes a helicase that operates at replication forks of the bacterium and certain of its bacteriophages to produce separated strands suitable for subsequent use by primase and DNA **polymerase** III. Here, we present the sequence of the dnaB gene of Salmonella typhimurium, a functionally interchangeable **analog** of the E. coli dnaB gene. The DnaB proteins of these two organisms, inferred from the DNA sequences, are identical in length and in 93% of amino acid

residues. Extended portions of the DnaB proteins are also similar to two phage-encoded DNA replication proteins: the gene 4 helicase-primase of coliphage **T7** and, as reported previously (H. Backhaus and J. B. Petri, Gene 32: 289-303, 1984), the gene 12 protein of Salmonella phage P22. In contrast, little similarity was found between DnaB and either the UvrD repair helicase or transcription termination factor Rho (an **RNA** -DNA helicase). These results identify S. typhimurium DnaB as a member of the DnaB family of proteins by structural, as well as functional, criteria and provide the basis for the eventual identification, by mutational studies, of residues in DnaB critical for its function.

5/3,AB/125 (Item 125 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05730785 88157716 PMID: 3279395
Accurate in vitro cleavage by RNase III of phosphorothioate-substituted **RNA** processing signals in bacteriophage **T7** early mRNA.
Nicholson A W; Niebling K R; McOsker P L; Robertson H D
Department of Biological Sciences, Wayne State University, Detroit, MI 48202.
Nucleic acids research (ENGLAND) Feb 25 **1988**, 16 (4) p1577-91, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM28294; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To test the ability of an **RNA** processing enzyme to cleave chemically-modified **RNA** substrates, **RNA** transcripts containing RNase III cleavage sites were enzymatically synthesized in vitro to contain specific phosphorothioate diester internucleotide linkages. One transcript (R1.1 **RNA**) was generated using phage **T7** **RNA** **polymerase** and a cloned segment of phage **T7** DNA containing the R1.1 RNase III processing site. The second transcript was the phage **T7** polycistronic early mRNA precursor, which was synthesized using E. coli **RNA** **polymerase** and **T7** genomic DNA. The **RNA** transcripts contained phosphorothioate diester groups at positions including the scissile bonds. The modified RNAs were stable to incubation in Mg2+-containing buffer, and were specifically cleaved by RNase III. **RNA** oligonucleotide sequence analysis showed that the modified R1.1 **RNA** processing site was the same as the canonical site and contained a phosphorothioate bond. Furthermore, RNase III cleaved the phosphorothioate internucleotide bond with 5' polarity. RNase III cleavage of phosphorothioate substituted **T7** polycistronic early mRNA precursor produced the same gel electrophoretic pattern as that obtained with the control transcript. Thus, RNase III cleavage specificity is not altered by phosphorothioate internucleotide linkages.

5/3,AB/126 (Item 126 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05688694 88122131 PMID: 3323886
Human acidic ribosomal phosphoproteins P0, P1, and P2: analysis of cDNA clones, in vitro synthesis, and assembly.
Rich B E; Steitz J A
Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510-8024.
Molecular and cellular biology (UNITED STATES) Nov **1987**, 7 (11) p4065-74, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: GM 26154; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
cDNA clones encoding three antigenically related human ribosomal phosphoproteins (P-proteins) P0, P1, and P2 were isolated and sequenced. P1 and P2 are **analogous** to Escherichia coli ribosomal protein L7/L12, and P0 is likely to be an **analog** of L10. The three proteins have a nearly identical carboxy-terminal 17-amino-acid sequence (KEESEESD(D/E)DMGFGLFD-COOH) that is the basis of their immunological cross-reactivity. The identities of the P1 and P2 cDNAs were confirmed by the strong similarities of their encoded amino acid sequences to published primary structures of the homologous rat, brine shrimp, and Saccharomyces cerevisiae proteins. The P0 cDNA was initially identified by translation of hybrid-selected mRNA and immunoprecipitation of the products. To demonstrate that the coding sequences are full length, the P0, P1, and P2 cDNAs were transcribed in vitro by bacteriophage **T7 RNA polymerase** and the resulting mRNAs were translated in vitro. The synthetic P0, P1, and P2 proteins were serologically and electrophoretically identical to P-proteins extracted from HeLa cells. These synthetic P-proteins were incorporated into 60S but not 40S ribosomes and also assembled into a complex similar to that described for E. coli L7/L12 and L10.

5/3,AB/127 (Item 127 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05669391 88087142 PMID: 3275650
Interaction of **T7 RNA polymerase** with DNA in an elongation complex arrested at a specific psoralen adduct site.
Shi Y B; Gamper H; Hearst J E
Department of Chemistry, University of California, Berkeley 94720.
Journal of biological chemistry (UNITED STATES) Jan 5 **1988**, 263 (1) p527-34, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM 11180; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have probed the interaction of **T7 RNA polymerase** with DNA in an elongation complex arrested by a site specifically placed psoralen diadduct or furanside monoadduct using DNase I footprinting techniques. The psoralen derivative, HMT (4'-hydroxy-methyl-4,5',8-trimethylpsoralen), was first placed at a specific site in the middle of a chemically synthesized double-stranded DNA fragment containing a **T7 RNA polymerase** promoter at one end. The psoralen molecule was photochemically attached either to 2 adjacent thymidine residues on opposite strands as a diadduct or to only 1 thymidine residue on the coding strand as a furan-side monoadduct. Using these psoralen-modified DNAs as templates for transcription, we found that **T7 RNA polymerase** was blocked at the psoralen adduct site and that the arrested elongation complex protected about 15 nucleotides upstream from the adduct on the coding strand and 20 nucleotides around the adduct on the noncoding strand from DNase I digestion. The two psoralen-modified DNA templates yielded identical **RNA** transcripts and DNase I footprints. In contrast, **T7 polymerase** protected only the coding strand from -20 to +8 in the initiation complex. These results suggest that the **RNA polymerase** undergoes a marked conformational change upon converting from an initiation complex to an elongation complex.

5/3,AB/128 (Item 128 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05665438 88096666 PMID: 3697141

Self-splicing of Tetrahymena rRNA can proceed with phosphorothioate substitution at the splice sites.
Deeney C M; Eperon I C; Potter B V
Department of Biochemistry, Leicester University, UK.
Nucleic acids symposium series (ENGLAND) **1987**, (18) p277-80, ISSN 0261-3166 Journal Code: 8007206
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The self-excision of a 413-base intervening sequence of the 26S rRNA of Tetrahymena thermophila has been investigated using phosphorothioate-substituted **RNA**. Transcripts containing this intron were prepared by **T7** **RNA** **polymerase** -catalyzed polymerisation using a M13 mICE10 vector in the presence of various nucleoside alpha-thiotriphosphate **analogues** . Wild-type transcripts incorporating phosphorothioates 5' to adenosine or uridine were inactive, whereas incorporation 5' to cytidine or guanosine allowed splicing. The first two substitutions place phosphorothioates inter alia at the 5' and 3' splice sites respectively. Mutagenesis at either site allowed phosphorothioate substitution 5' to guanosine at each splice site. This did not block splicing, suggesting that substitution at internal sites within the intron has more effect.

5/3,AB/129 (Item 129 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05505961 87257810 PMID: 3299044
Kinetics of inhibition by 8-oxy-ATP of the dinucleotide pppApU synthesis catalyzed by Escherichia coli **RNA-polymerase** on the promoter A1 of phage **T7** delta D111 DNA during coupled synthesis of di- and trinucleotides and a limited set of substrates]
Kinetika ingibirovaniia 8-oksi-ATP sinteza dinukleotida pppApU RNK-polimerazoi Escherichia coli na promotore A1 DNK faga **T7** delta D111 v usloviiakh sopriazhennogo sinteza di- i trinukleotida i ogranichennogo nabora substratov.
Kuriavyi V V; Bruskov V I
Molekuliarnaia biologiia (USSR) Mar-Apr **1987**, 21 (2) p462-71, ISSN 0026-8984 Journal Code: 0105454
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
A kinetic analysis of inhibition of synthesis of dinucleotide pppApU catalyzed by Escherichia coli **RNA-polymerase** on A1 promoter of the DNA from **T7** delta DIII phage mutant by 8-oxy-ATP under the conditions of the coupled synthesis of pppApU and pppApUpC and in the presence of an incomplete set of substrates, namely ATP, UTP, CTP, has been performed. It was found that 8-oxy-ATP is an unproductive **analog** of both ATP and CTP. A comparative analysis of the dissociation constants shows that 8-oxy-ATP binds at ATP center 3.3. times and at CTP center 540 times weaker than natural substrates. At the UTP center 8-oxy-ATP does not bind at all.

5/3,AB/130 (Item 130 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05500562 87250553 PMID: 3036847
Purified **RNA** **polymerase** II recognizes specific termination sites during transcription in vitro.
Dedrick R L; Kane C M; Chamberlin M J
Journal of biological chemistry (UNITED STATES) Jul 5 **1987**, 262

(19) p9098-108, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have studied the ability of certain well-defined prokaryotic DNA sequences to act as specific termination signals for highly purified calf thymus **RNA polymerase** II. We used duplex DNA fragments modified to direct efficient and specific transcription of defined DNA templates to follow transcription with **RNA polymerase** alone in the absence of additional protein factors. Elongation of **RNA** chains by **RNA polymerase** II is processive through most DNA sequences. However, certain DNA sequences serve as effective "intrinsic" terminators for **RNA polymerase** II; in this they resemble the "rho-independent" terminators for the bacterial **RNA polymerase** . Several rho-independent bacterial terminators are also able to act as termination signals for **RNA polymerase** II. However, there is no apparent correlation between the efficiency of termination for the bacterial enzyme and that found for the calf thymus enzyme. One very efficient bacterial terminator (phage **T7** early terminator) gives no termination with **RNA polymerase** II, and we have identified at least two sites that cause the eukaryotic enzyme to terminate but have no effect on transcription by the bacterial enzyme. Hence, the signals recognized as intrinsic termination sites for the two enzymes are substantially different. All of the sites that act as intrinsic terminators for **RNA polymerase** II contain a series of consecutive thymidine residues in the nontranscribed DNA strand (T-run), and the 3' end of the completed **RNA** normally lies within this sequence. It is plausible that the T-run is part of the signal for an **RNA polymerase** II termination site; however, there is no apparent correlation between the number of T residues and the efficiency of the terminator, suggesting that other sequence elements are required for, or modulate, termination. Several lines of evidence suggest that the formation of **RNA** secondary structures in the nascent transcript is not an essential element of the intrinsic **RNA polymerase** II termination signal.

5/3,AB/131 (Item 131 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05487007 87242326 PMID: 3036203
Interactions of **T7 RNA polymerase** with **T7** late promoters measured by footprinting with methidiumpropyl-EDTA-iron(II).
Gunderson S I; Chapman K A; Burgess R R
Biochemistry (UNITED STATES) Mar 24 **1987**, 26 (6) p1539-46, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: 5 T32 GM07133; GM; NIGMS; CA07175; CA; NCI; CA23076; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The interactions of **T7 RNA polymerase** with **T7** late promoters were studied by using quantitative footprinting with methidiumpropyl-EDTA X Fe(II) [MPE-Fe(II)] as the DNA cleaving agent. Class II and class III **T7** promoters have a highly conserved 23 base pair sequence from -17 to +6. Among class III promoters the -22 to -18 region is also highly conserved. For a class II promoter, **T7 RNA polymerase** protects the -17 to -4 region from MPE-Fe(II) cleavage; when GTP is present, protection extends from -17 to +5 (noncoding strand). For a class III promoter, protection extends from -20 to -4 and in the presence of GTP from -20 to +5 (noncoding strand). The protected regions for the coding strands of both promoters were nearly identical with that seen for the noncoding strands. The binding constant for the class III

promoter is (4 +/- 1.5) X 10(7) M-1 and in the presence of GTP increases to (10 +/- 1.7) X 10(7) M-1. These binding constants are about 1000 and 200 times greater, respectively, than values reported previously [Ikeda, R. A., & Richardson, C. C. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 3614-3618]. The differences in binding constants are probably due to tRNA and high salt used in those earlier experiments. Both tRNA and high salt (greater than 50 mM NaCl and greater than 10 mM MgCl2) inhibit the binding of the **polymerase** to the promoter. Optimal binding conditions occur at 2-5 mM MgCl2 and 0-10 mM NaCl.(ABSTRACT TRUNCATED AT 250 WORDS)

5/3,AB/132 (Item 132 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05478503 87230996 PMID: 2438652
Stereospecificity of nucleases towards phosphorothioate-substituted **RNA**: stereochemistry of transcription by **T7 RNA polymerase**.
Griffiths A D; Potter B V; Eperon I C
Nucleic acids research (ENGLAND) May 26 **1987**, 15 (10) p4145-62
, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Transcription by **T7 RNA polymerase** has been studied using a chiral ATP **analogue** . The Sp diastereoisomer of adenosine 5'-O-(1-thiotriphosphate) (ATP alpha S) was incorporated into **RNA** with an apparent KM of approximately 15 microM, similar to that for ATP; the Rp diastereoisomer was neither a substrate nor a competitive inhibitor. The configuration of the phosphodiester link in the **RNA** produced was analyzed with stereospecific nucleases. The rate of nuclease digestion was compared with the rate of digestion of phosphorothioate-substituted **RNA** of known stereochemistry synthesized by E. coli **RNA polymerase** . Surprisingly, the nucleases exhibited reduced discrimination compared with their activity on dinucleotides. The results show that phosphorothioate-substituted **RNA** transcribed by **T7 RNA polymerase** has the same configuration as that transcribed by E. coli **RNA polymerase**, ie. Rp. Thus, the reaction proceeds with inversion of configuration at phosphorus.

5/3,AB/133 (Item 133 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05478496 87230989 PMID: 2438651
Single base bulges in small **RNA** hairpins enhance ethidium binding and promote an allosteric transition.
White S A; Draper D E
Nucleic acids research (ENGLAND) May 26 **1987**, 15 (10) p4049-64
, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: CA01081; CA; NCI; GM37005; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A set of four **RNA** hairpin helices has been prepared by in vitro transcription with **T7 RNA polymerase**. The hairpins all contain the same nine base pair helix, but with an extra A, C, or U residue forming a bulge at one position; the fourth hairpin is a perfect helix with no bulge. The helix with a bulged A duplicates six base pairs of a helix in the 16S rRNA known to have an unusually high affinity for ethidium bromide [J. M. Kean, S. A. White, and D. E. Draper, Biochemistry 24, 5062 (1985)]. Binding and chemical cleavage studies with ethidium or the reagent

methidiumpropylEDTA-Fe(II) [MPE-Fe(II)] showed that the sequence CpG is a preferred intercalation site whether or not a bulge is present; all three bulged bases enhance intercalation at the CpG sequence by an order of magnitude; and intercalation in a bulged helix results in a concerted conformational change involving the entire helix backbone, again dependent on the presence of a bulge but independent of the particular base. These results suggest that an extra sugar-phosphate residue in an **RNA** helix backbone has a dramatic effect on the ability of the **RNA** to adopt new conformations. This effect could be an important reason for the conservation of bulges at certain positions in ribosomal and other RNAs.

5/3,AB/134 (Item 134 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05379953 87129098 PMID: 2434144
Effect of 8-Br-ATP and 8-Oxy-ATP on **RNA** synthesis by **RNA polymerase** from Escherichia coli]
Vliianie 8-Br-ATP i 8-Oxy-ATP na sintez RNK RNK-polimerazoi Escherichia coli.
Kuriavyi V V; Bruskov V I
Biokhimii a (Moscow, Russia) (USSR) Jan **1987**, 52 (1) p138-41, ISSN 0320-9725 Journal Code: 0372667
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
The effect of 8-Br-ATP and 8-oxy-ATP on **RNA** synthesis on calf thymus DNA and on abortive synthesis of di- and trinucleotides on promoter AI of phage **T7** delta DIII DNA in the case of an incomplete set of substrates was studied. It was shown that the ATP **analogs** used inhibit the **RNA** and di- and trinucleotide synthesis. In all cases, 8-oxy-ATP was a more effective inhibitor than 8-Br-ATP. Both **analogs** are incapable of being the primer and they do not replace ATP in the course of abortive initiation of pppApU synthesis.

5/3,AB/135 (Item 135 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05297548 87049601 PMID: 3535875
An Escherichia coli **RNA polymerase** tight-binding site on **T7** DNA is a weak promoter subject to substrate inhibition.
Prosen D E; Cech C L
Biochemistry (UNITED STATES) Sep 23 **1986**, 25 (19) p5378-87, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A specific Escherichia coli **RNA polymerase** tight-binding (TB) site on bacteriophage **T7** has been located at 32,988 base pairs from the left end of **T7**. This site is referred to as the **T7** F promoter since it is fully active in vitro. Kinetics of association and dissociation have been measured by use of the abortive initiation assay and runoff transcription. The association constant, ka approximately 9 X 10(5) M-1 s-1, is of the same magnitude as ka for the **T7** minor promoters. In competitive titration assays, the F promoter was found to be slightly weaker than the minor **T7** E promoter at low **RNA polymerase** concentrations and, as expected, much weaker than the major **T7** A3 promoter. An unusual **RNA polymerase** mediated inhibition of both the association rate and the transcriptional activity was observed at moderately high concentrations of **polymerase**. A mechanistic model **analogous** to enzyme substrate inhibition is presented.

5/3,AB/136 (Item 136 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05273989 87026583 PMID: 3094579
Structural and functional differences between the two intrinsic zinc ions of Escherichia coli **RNA polymerase**.
Giedroc D P; Coleman J E
Biochemistry (UNITED STATES) Aug 26 **1986**, 25 (17) p4969-78, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: AM09070-21; AM; NIADDK; GM10972; GM; NIGMS; GM21919-11; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
DNA-dependent **RNA polymerase** (RPase) from Escherichia coli contains 2 mol of intrinsic Zn(II)/mol of core enzyme (alpha 2 beta beta'). In techniques **analogous** to those employed with the Zn(II) metalloenzyme aspartate transcarbamoylase [Hunt, J. B., Neece, S. H., Schachman, H. K., & Ginsberg, A. (1984) J. Biol. Chem. 259, 14793-14803], we show that titration of core or holoRPase with 10 or 16 equiv, respectively, of the sulfhydryl reagent p-(hydroxymercuri)benzenesulfonate (PMPS) results in the facile release of 1 mol of Zn(II) [B-site Zn(II)] in a reaction totally reversible with the addition of excess thiol provided no metal chelator is present. If ethylenediaminetetraacetic acid (EDTA) is present, reversal of the PMPS-enzyme complex results in formation of a Zn1 RPase [A-site Zn(II)]. This enzyme retains full transcriptional activity relative to Zn2 RPase on both calf thymus (nonspecific) and **T7** (sigma-dependent, specific) DNA templates. If the core enzyme-PMPS complex is incubated with a large excess of another metal such as Cd(II) followed by thiol treatment, a hybrid ZnACdB RPase is formed. Direct treatment of the enzyme with excess Cd(II) also gives rise to a hybrid ZnACdB RPase. Transcription by these enzymes is also comparable to that of the starting Zn2 enzyme. Isolation of in vivo synthesized Co2 RPase and Cd2 RPase and treatment of either enzyme with PMPS/EDTA results in formation of a CoA and CdA enzyme, respectively. Co(II)A and Cd(II)A enzymes show 123 and 76%, respectively, of the elongation rates on **T7** DNA observed for the Zn(II) enzyme. Visible absorption spectroscopy of the Co2 enzyme exhibits four d-d transition bands positioned at 760 (epsilon = 800), 710 (epsilon = 900), 602 (epsilon = 1500), and 484 (epsilon = 4000) nm. In addition, two charge-transfer bands are found at 350 (epsilon = 9600) and 370 (epsilon = 9500) nm. Only the Co(II) ion bound at site A is associated with this unique set of intense d-d transitions. The positions and intensities of both the visible and charge-transfer bands of Co(II)A RPase approximate those shown by Co(II)-substituted metalloenzyme sites where the ligands are four S rather than mixed S,N or S,O sites.

5/3,AB/137 (Item 137 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05105826 86182444 PMID: 3938589
Substrate selection by **RNA polymerase** from E. coli. The role of ribose and 5'-triphosphate fragments, and nucleotides interaction.
Szafranski P; Smagowicz W J; Wierzchowski K L
Acta biochimica Polonica (POLAND) **1985**, 32 (4) p329-49, ISSN 0001-527X Journal Code: 14520300R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Steady-state kinetic studies of the rifampicin-effected abortive

initiation of transcription by E. coli **RNA polymerase** (EC 2.7.7.6) on the A1 **T7** phage promoter were carried out with the use of ATP, UTP and a number of their appropriately modified **analogues**. The kinetic parameters KiA, KmB, Ki and KsB characterizing the affinity of the substrates and inhibitors of the reaction to the initiation and elongation sites of the enzyme:promoter and the enzyme:promoter:nucleoside triphosphate complexes were determined therefrom. Their comparative analysis indicated that 1) the triphosphate chain of the initiating purine nucleoside triphosphate interacts with some protein acceptor groups through the alpha- and beta-phosphate residues; the phosphates are engaged in binding of nucleoside triphosphates at the elongation site in the absence of the primer nucleotide; 2) the ribose 2'-OH of the elongating nucleotide, but neither of the ribose hydroxyl groups of the initiating nucleotide, participate in substrate recognition by protein receptors; 3) either substrate, ATP or UTP, bound to the initiation complex increases by about the same factor (greater than or equal to 10) the affinity of the other to its binding site; 4) the 3'-OH of the primer nucleotide and the gamma-phosphate of the elongating nucleotide are involved in the synergistic interaction of the substrates; alpha- and beta-phosphates of the elongating nucleotide, bound to some protein receptors, also contribute to this process. It is postulated that the interaction of substrates is mediated through an Mg2+ ion, known to be required for binding of the substrates in the elongation site, and a minimal molecular model of a PuoTP:Mg (II): nucleoside triphosphate chelate complex in the catalytic centre of the transcription initiation open complex is proposed.

5/3,AB/138 (Item 138 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05025890 86104052 PMID: 3002422
Transcription from bacteriophage **T7** and SP6 **RNA polymerase** promoters in the presence of 3'-deoxyribonucleoside 5'-triphosphate chain terminators.
Axelrod V D; Kramer F R
Biochemistry (UNITED STATES) Oct 8 **1985**, 24 (21) p5716-23, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM-33345; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
RNA synthesis by **T7 RNA polymerase** or SP6 **RNA polymerase** is 100-1000 times more sensitive to the presence of the 3'-deoxyribonucleoside 5'-triphosphate chain terminators than is **RNA** synthesis by Escherichia coli **RNA polymerase** or Q beta replicase. These ribonucleotide **analogues** do not alter the specificity of each **polymerase** for its own promoters nor do they alter the site at which synthesis is initiated. Transcription by **T7 RNA polymerase** or SP6 **RNA polymerase** in the presence of relatively low concentrations of these chain terminators offers a useful route for determining the nucleotide sequence of any DNA segment that is inserted immediately downstream from a homologous bacteriophage promoter. This sequencing procedure was used to explore the effects that different dinucleotides have on the specificity of initiation at two different **T7 RNA polymerase** promoters.

5/3,AB/139 (Item 139 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03823839 82103706 PMID: 6275257
Pyrophosphate **analogs** in the pyrophosphorolysis reaction catalyzed by Escherichia coli **RNA polymerase**]

Analogi pirofosfata v reaktsii pirofosforoliza, kataliziruemoi RNK-polimerazoi Escherichia coli.
Rozovskaia T A; Chenchik A A; Tarusova N B; Bibilashvili R Sh; Khomutov R M
Molekuliarnaia biologiia (USSR) Nov-Dec **1981**, 15 (6) p1205-23, ISSN 0026-8984 Journal Code: 0105454
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
Processive pyrophosphorolysis of **RNA** from ternary **RNA polymerase**-nascent **RNA**-delta D111 **T7** DNA complex has been followed in the absence of nucleoside triphosphates. Series of inorganic pyrophosphate **analogs** were investigated for their ability to sustain the reaction and to compete with inorganic pyrophosphate for the reaction. Methylenediphosphonic, imidodiphosphonic, phosphonacetic acids, inorganic triphosphate, methylenediphosphonic and phosphate were found to be capable of substituting the inorganic pyrophosphate in **RNA** degradation reaction with tantamount efficiency. They give rise to nucleoside monophosphates for phosphonoacetic acid, nucleoside triphosphates for inorganic pyrophosphate and inorganic triphosphate, nucleoside triphosphates **analogs** for methylenediphosphonic, imidodiphosphonic acids and methylenediphosphonic acid phosphate as the low molecular weight product of the reaction. The problem of specific interaction of **RNA polymerase** with nucleoside triphosphates and inorganic pyrophosphate is discussed in the terms of structural requirements for the compounds to be a potent substrate for **RNA polymerase**.

5/3,AB/140 (Item 140 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03708838 81246848 PMID: 7019855
The properties of ATP-**analogs** in initiation of **RNA** synthesis catalyzed by **RNA polymerase** from E coli.
Smagowicz W J; Scheit K H
Nucleic acids research (ENGLAND) May 25 **1981**, 9 (10) p2397-401, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Various base and sugar modified derivatives of ATP and UTP were used as substrate **analogs** for the steady state initiation reaction ATP+UTP=pppApU and the single step addition reaction ApC+ATP=ApCpA. These reactions were carried out by E. coli **RNA polymerase** on **T7** DNA in the presence of rifampicin. The steady state kinetic parameters of the **analogs**, either as substrates or inhibitors, were determined. On the basis of the obtained results it is concluded that purine NTP s in initiation require anti-conformation about the glycosidic bonds as well as gauche-gauche conformation of the C(4')-C(5') bonds. The latter conformation is also a prerequisite for substrates in elongation, whereas strict anti-conformation of glycosidic bonds is not.

5/3,AB/141 (Item 141 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03209125 80020927 PMID: 226121
Mechanistic studies on deoxyribonucleic acid dependent ribonucleic acid **polymerase** from Escherichia coli using phosphorothioate **analogues**. 1. Initiation and pyrophosphate exchange reactions.
Yee D; Armstrong V W; Eckstein F
Biochemistry (UNITED STATES) Sep 18 **1979**, 18 (19) p4116-20,

ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The diastereomers of adenosine 5'-O-(1-thiotriphosphate) (ATP alpha S) and adenosine 5'-O-(2-thiotriphosphate) (ATP beta S) can replace adenosine triphosphate (ATP) in the initiation reaction catalyzed by deoxyribonucleic acid (DNA) dependent ribonucleic acid (**RNA**) **polymerase** from Escherichia coli. In both cases, the Sp diastereomer is a better initiator than the Rp isomer. The diasteromers of 3'-uridyl 5'-adenosyl ,O-phosphorothioate [Up(S)A] can replace UpA in the primed initiation reaction catalyzed by **RNA polymerase** ; however, the Rp diastereomer is a better initiator than the Sp isomer. By using ATP or CpA as initiator and UTP alpha S, isomer A, as substrate, we determined the stereochemical courses of both the initiation and primed initiation reactions, respectively, with **T7** DNA template and found them to proceed with inversion of configuration. Determination of the stereochemical course of the pyrophosphate exchange reaction catalyzed by **RNA polymerase** provides evidence that this reaction is the reverse of the phosphodiester bond-forming reaction.

5/3,AB/142 (Item 142 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03006181 79074236 PMID: 724495
Specific termination of **RNA polymerase** synthesis as a method of **RNA** and DNA sequencing.
Axelrod V D; Vartikyan R M; Aivazashvili V A; Beabealashvili R S
Nucleic acids research (ENGLAND) Oct **1978**, 5 (10) p3549-63, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Termination of **RNA** synthesis with 3'-O-Methylnucleoside 5'-triphosphates have been studied using E. coli **RNA polymerase** holoenzyme and poly [d(A-T)] as well as unfractionated **T7** D delta III DNA as templates. It was shown that the termination can be used for DNA sequencing. A sequence of a part of **RNA** synthesized from AI promoter of the DNA have been determined. Syntheses of four 3'-O-Methylnucleoside 5'-triphosphates are described.

5/3,AB/143 (Item 143 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02893380 78218144 PMID: 353045
Transcription of **T7** DNA containing modified nucleotides by bacteriophage **T7** specific **RNA polymerase**.
Stahl S J; Chamberlin M J
Journal of biological chemistry (UNITED STATES) Jul 25 **1978**, 253 (14) p4951-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The interaction of bacteriophage **T7** specific **RNA polymerase** with its cognate promoter sites has been probed by selectively replacing bases in one **T7** promoter site with base **analogs**. Base **analogs** such as 2,6-diaminopurine or hypoxanthine, which alter residues appearing in the minor groove of the DNA helix, prevent utilization of the promoter by **T7** **RNA**

polymerase. These **analogs** do not affect transcription which starts outside of the modified region. In contrast, base **analogs** that have alterations that appear in the major groove of the DNA helix, such as uracil, 5-bromouracil, 5-methylcytosine, 5-hydroxymethylcytosine, and [5-HgSR]pyrimidines, do not prevent utilization of the promoter. The deoxyribonucleoside **analog** 5'-imino-5'-deoxythymidine, an alteration appearing in the deoxyribose-phosphodiester backbone of the DNA helix, does not prevent promoter recognition. Haemophilus aegyptius restriction endonuclease III, which cleaves DNA at the sequence 5'GGCC3', does not act at sites in which the guanine residues in one of the two DNA strands have been substituted with hypoxanthine. This implicates the guanine amino group in the minor groove of the DNA helix as a possible recognition point for this restriction endonuclease.

5/3,AB/144 (Item 144 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02852566 78168929 PMID: 348467
A study of unwinding of DNA and shielding of the DNA grooves by **RNA polymerase** by using methylation with dimethylsulphate.
Melnikova A F; Beabealashvilli R; Mirzabekov A D
European journal of biochemistry / FEBS (GERMANY, WEST) Mar **1978**, 84 (1) p301-9, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The dimethylsulphate method has been used to study the complexes of **RNA polymerase** (Escherichia coli) with DNA of **T7** phage, poly[d(A--T)] and fragments of calf thymus DNA protected against DNase digestion by **RNA polymerase**. The binding of **RNA polymerase** to DNA significantly increases the formation of 1-methyl-adenine produced by methylation of the single-stranded DNA region, diminishes by about 10% the formation of 3-methyl-adenine by methylation within the minor groove and does not affect the formation of 7-methyl-guanine by methylation within the major DNA groove. The presence of nascent **RNA** decreases the formation of 1-methyl-adenine in DNA of the complex by about 30%. The initiation of **RNA** synthesis or **RNA** synthesis itself does not influence the methylation of the major groove but shielding of the minor groove increases by about twice as much. These results suggest that **RNA polymerase**, upon binding, breaks Watson-Crick base-pairing in a DNA region of about 15-base-pairs long, that nascent **RNA** forms a duplex with DNA of about 10-base-pairs long; and that the enzyme weakly interacts with DNA along its grooves and preferentially makes contacts with the minor groove.

5/3,AB/145 (Item 145 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02313273 76165279 PMID: 1261543
The interdependence of magnesium with spermidine and phosphoenolpyruvate in an enzyme-synthesizing system in vitro.
Fuchs E
European journal of biochemistry / FEBS (GERMANY, WEST) Mar 16 **1976**, 63 (1) p15-22, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The DNA-dependent syntheses of different enzymes of the bacteriophages T3 and **T7** were studied in an Escherichia coli system in vitro with respect to the optimal Mg2+ concentration and its interdependence with

substituting (e.g. spermidine) and complexing agents (e.g. phosphoenolpyruvate). The following results were obtained. 1. The optimal conditions for the syntheses of the different enzymes were not identical. The optima for **RNA polymerase** synthesis were 8 mM Mg2+, 10 mM P-pyruvate and 3 mM spermidine; for S-adenosyl-L-methionine cleaving enzyme synthesis, 6 mM Mg2+, 6 mM P-pyruvate and 3 mM spermidine; and for lysozyme synthesis, 13-18 mM Mg2+, 28 mM P-pyruvate and 3-0 mM spermidine. 2. The optimal conditions for the synthesis of **analog** enzymes (**RNA** polymerases and lysozymes) from the two templates were identical with experimental error. 3. Mg2+ and spermidine substituted for each other in relation to the number of their charges. 4. The apparent complexing of one Mg2+ molecule required the addition of 3-5 P pyruvate molecules. 5. Under the optimal conditions the enzyme-synthesizing activity was higher by more than a factor of 10 compared to previously described systems.

5/3,AB/146 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10718591 BIOSIS NO.: 199799339736
Specific and nonspecific inhibition of transcription by DNA PNA, and phosphorothioate promoter **analog** duplexes.
AUTHOR: Hamilton Susan E; Iyer Mridula; Norton James C; Corey David R(a)
AUTHOR ADDRESS: (a)Howard Hughes Med. Inst., Dep. Pharmacology; Univ. Texas Southwestern Med. Cent. at Dallas, 5323**USA
JOURNAL: Bioorganic & Medicinal Chemistry Letters 6 (23):p2897-2900 **1996**
ISSN: 0960-894X
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: DNA duplexes **analogous** to the promoters for SP6 or **T7 RNA polymerase** inhibit transcription with exquisite selectivity. By contrast, phosphorothioate oligomers inhibit nonselectively, and peptide nucleic acid (PNA) duplexes and PNA:DNA heteroduplexes do not inhibit at all. The absence of recognition of proteins by PNAs may prove to be a substantial advantage for their use as anti-sense agents and nucleic acid probes.

1996

5/3,AB/147 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09666542 BIOSIS NO.: 199598121460
Inhibition of **T7 RNA polymerase** transcription by phosphate and phosphorothioate triplex-forming oligonucleotides targeted to a R cntdot Y site downstream from the promoter.
AUTHOR: Alunni-Fabbroni Marianna; Manfioletti Guidalberto; Manzini Giorgia; Xodo Luigi E(a)
AUTHOR ADDRESS: (a)Dep. Biochem., Biophys. Macromol. Chem., Via Giorgieri 1, Univ. Trieste, I-34127 Trieste**Italy
JOURNAL: European Journal of Biochemistry 226 (3):p831-839 **1994**
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The effect of triplex-forming oligonucleotides (TFO) on the transcription activity of **T7 RNA polymerase** has been investigated by an in vitro assay. The TFOs, either containing only

phosphate (PO-2) or phosphate and phosphorothioate (POS) internucleotide linkages, were targeted to a 30-bp homopurine cntdot homopyrimidine (R cntdot Y) site cloned in plasmid Bluescript KS+ about four helical turns downstream from the **T7 RNA** promoter. Band-shift and ultraviolet absorption melting experiments showed that the designed pyrimidine PO-2 and POS TFOs form stable triple-helical complexes with the R cntdot Y target duplex (the DELTA-G-TFO values of triplex formation vary from -42 to -63 kJ/mol). The triple-helical complexes resulting from POS oligonucleotides were less stable (by 4-12 kJ/ mol) than those obtained with PO-2 **analogues**, the magnitude of destabilization being dependent on the number of POS groups present in the third strand. The designed TFOs were shown to efficiently repress bacteriophage **T7 RNA polymerase** transcription under different experimental conditions. The repression depended on pH, TFO concentration and temperature. When the TFO/template ratio was fixed to 100, a strong repressive effect was observed with normal and phosphorothioate pyrimidine TFOS, also under physiological conditions. In contrast, a purine-rich oligonucleotide containing 44% of guanine residues promoted only a weak transcription inhibition, even at a TFO/template ratio as high as 750. Both PO-2 and POS-containing pyrimidine TFOs produced their strong repressive effect on **T7 RNA polymerase** transcription even when they were added to the reaction mixture simultaneously with the **polymerase**. A mechanism of transcription repression is discussed. The data reported in this paper are useful for designing oligonucleotides acting as artificial repressors in the antigene strategy and indicate that the R cntdot Y target need not to be precisely confined to the promoter.

1994

5/3,AB/148 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08991617 BIOSIS NO.: 199396143118
Preparation of infectious Venezuelan equine encephalitis virus based on full length DNA copy of its genome.
AUTHOR: Kolykhalov A A; Frolov I V; Agapov E V; Netesov S V; Sandakhchiev L S
AUTHOR ADDRESS: Res. Inst. Mol. Biol., Sci. Prod. Assoc. "Vector", Koltsovo, Novosibirsk**Russia
JOURNAL: Doklady Akademii Nauk 327 (1):p160-164 **1992**
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian; Non-English

ABSTRACT: An attempt was made to create an efficient experimental system to study the effect of mutation-induced changes in various genes on the biological properties of Venezuelan equine encephalitis (VEE) virus and first of all on its attenuation. Experiments were carried out with a Trinidad donkey strain of VEE virus. Plasmid pVE-57 constructed contained full-length DNA-copy of genome. The plasmid was then used for the synthesis of **RNA analogue** of VEE genome in in-vitro transcription reaction, using **RNA-polymerase** of phase **T7**. The results of the transfection of the cell culture by **RNA** preparations obtained under varying transcription conditions were presented. The biological properties of the VEE virus obtained on the basis of the genome copy and the initial strain were compared in mice. It was found that the viruses were identical. Molecular sequence data are presented.

1992

5/3,AB/149 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08935486 BIOSIS NO.: 199396086987
Rescue of synthetic **analogs** of genome **RNA** of human parainfluenza virus type 3.
AUTHOR: De Bishnu P(a); Banerjee Amiya K
AUTHOR ADDRESS: (a)Dep. Molecular Biol., Res. Inst., Cleveland Clinic Found., Cleveland, OH 44195**USA
JOURNAL: Virology 196 (1):p344-348 **1993**
ISSN: 0042-6822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A simple system that allows expression and packaging of a foreign gene by human parainfluenza virus type 3 (HPIV-3) has been described. First, a cDNA was constructed to encode an internally deleted version of HPIV-3 genome **RNA**. The viral genes were replaced with a negative sense copy of the bacterial chloramphenicol acetyl transferase (CAT) reporter gene. In vitro run-off transcription with **T7 RNA polymerase** synthesized an 870 nucleotide **RNA** that contained the antisense coding region of the CAT gene flanked by the transcription regulatory sequences and the 3' and 5' end extracistronic sequences of the HPIV-3 genome. When introduced into cells that are infected with HPIV-3, this **RNA** was amplified and the reporter gene was expressed, as measured by the CAT activity in the cell extract. Furthermore, the synthetic **RNA** was packaged into infectious virions. The addition of two extra nucleotides at the 5' end of the parental trailer region decreased the CAT activity by more than 90%, suggesting a requirement for the intact 5'-regulatory domain in the viral replicative cycle. Interestingly, the addition of one extra nucleotide to the 3' end totally abolished the CAT activity indicating that an exact 3' terminus is critical in this process.

1993

5/3,AB/150 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08923607 BIOSIS NO.: 199396075108
Bacteriophage **T7 RNA polymerase**: Fluorine-19 nuclear magnetic resonance observations at 5-fluorouracil-substituted promoter DNA and **RNA** transcript.
AUTHOR: Rastinejad Fraydoon; Lu Ponzy(a)
AUTHOR ADDRESS: (a)Dep. Chem., Univ. Pa., Philadelphia, PA 19104**USA
JOURNAL: Journal of Molecular Biology 232 (1):p105-122 **1993**
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have substituted 5-fluorodeoxyuridine (5-FdU) in place of thymidine in defined positions along synthetic bacteriophage **T7** promoter DNA sequences. None of the fluoro-substitutions in the promoter DNA sequence reduced transcription yields with **T7 RNA polymerase** significantly. Substitutions on the coding template strand reduced transcription yields when placed at +3, but not at +4. 19F-n.m.r. spectra from transcription reactions and, gel analysis of transcription products show that **T7 RNA polymerase**

correctly and efficiently utilizes 5-FUTP as a **RNA** substrate **analog**. The fluorine atom provides a sensitive probe for monitoring the local environment, base sequence and solvent exposure at the DNA major groove through its 19F-n.m.r. resonance. Buffer dependencies of the fluorine chemical shift and digestion patterns with DNase I suggest that the **T7** promoter base-pairs near the transcription start site are distorted with a more open minor groove and less solvent accessible major groove. Previous chemical footprinting data of promoter-**polymerase** complexes yield a picture that **T7 RNA** minor groove features on the same side of DNA flanking both sides of this region. Consistent **polymerase** recognizes major groove features in the region from positions -7 to -11 and minor groove features on the same side of DNA flanking both sides of this region. Consistent with this, 19F-n.m.r. observations identify two additional positions, -8 and -17, involved in promoter recognition on this side of the DNA helix. On the other hand, our observations also implicate the opposite side of the DNA helix, primarily at positions -14 and -15, as major groove recognition sites for **T7 RNA polymerase**. In addition, n.m.r. spectra from 5-FdU-substituted base-pairs -2 and -3, suggest either additional interactions on the same side of the DNA helix as -14 and -15, or distortions in the DNA structure.

1993

5/3,AB/151 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08887970 BIOSIS NO.: 199396039471
Synthesis and characterization of a new photocrosslinking CTP **analog** and its use in photoaffinity labeling Escherichia coli and **T7** **RNA** polymerases.
AUTHOR: Hanna Michelle M; Zhang Yuying; Reidling Jack C(a); Thomas Matthew J; Jou Jerry
AUTHOR ADDRESS: (a)Dep. Biol. Chem., Univ. California, Irvine, CA**USA
JOURNAL: Nucleic Acids Research 21 (9):p2073-2079 **1993**
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A new photocrosslinking CTP **analog** that functioned as a substrate during transcription was synthesized and used to photoaffinity label E. coli and bacteriophage **T7 RNA** polymerases. This **analog**, 5-((4-azidophenacyl)thio) cytidine-5'-triphosphate (5-APAS-CTP) contains an aryl azide group approximately 10 ANG from the nucleotide base and specifically replaced CTP during synthesis of **RNA** by both polymerases. **Analog** was placed at the 3' end or internally within **RNA**. Both polymerases inefficiently incorporated two 5-AP AS-CMP molecules sequentially, as was found for the related 5-APAS-UMP. **Analog** was placed at the 3' end of **RNA** in transcription complexes paused at the site of Q-modification of E. coli **RNA polymerase**, downstream of the lambda PR' promoter (+ 16), a pause that requires specific DNA sequences but no apparent **RNA** hairpin. Crosslinking was examined in the presence and absence of the NusA protein, which enhances the transcriptional pause at this site and is required for Q modification of the **polymerase**. Crosslinking of the 3' end of the **RNA** to NusA was not observed, consistent with our earlier results involving a NusA-enhanced pause site downstream from an **RNA** hairpin.

1993

5/3,AB/152 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08875612 BIOSIS NO.: 199396027113
A study of the bacteriophage **T7** DNA-dependent **RNA polymerase** using GTP **analogs**.
AUTHOR: Mishin A A(a); Khropov Yu V; Tunitskaya V L; Kochetkov S N
AUTHOR ADDRESS: (a)V.A. Engel'gardt Inst. Mol. Biol., Acad. Sci. Russ., Moscow**Russia
JOURNAL: Biokhimiya 58 (1):p43-49 **1993**
ISSN: 0320-9725
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian; Non-English
SUMMARY LANGUAGE: Russian; English

ABSTRACT: Interactions of the bacteriophage **T7** DNA-dependent **RNA polymerase** with three GTP **analogs** have been studied. All of the three **analogs** tested contained substituted naphtalenesulphamide groups and were shown to be under appropriate conditions irreversible covalent inhibitors of the enzyme, the modified enzyme possessing fluorescent properties. One of these **analogs** contained the reactive 2-bromethyl phosphonate group and was shown to cause the loss of the enzyme affinity for polynucleotide templates. The other two modifiers which contained the azide reactive group did not alter the enzyme-template affinity, the polynucleotide binding leading to a notable increase of the enzyme fluorescence intensity. The latter two modifiers are supposed to be convenient for fluorescent labelling of the active site of **RNA polymerase** for enzyme-template binding studies.

1993

5/3,AB/153 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07998679 BIOSIS NO.: 000093054352
SYNTHESIS AND DETERMINATION OF THE BIOLOGICAL ACTIVITIES OF YEAST ALANINE TRNA **ANALOGUES**
AUTHOR: LU J-H; WANG D-B
AUTHOR ADDRESS: DEP. BIOL. CHEM., COLL. MED., UNIV. CALIF., IRVINE, CALIF. 92717, USA.
JOURNAL: SCI CHINA SER B CHEM LIFE SCI EARTH SCI 34 (10). 1991. 1198-1204.
1991
CODEN: SCBSE
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The role of base modification in yeast tRNAAla function in protein synthesis was examined by the use of unmodified tRNA **analogues**. Unmodified full length tRNAs, 5'-half tRNAs (nucleotides 1-35) and 3'-half tRNAs (nucleotides 37-75) were transcribed in vitro using **T7-RNA polymerase**. Unmodified tRNA half molecules were joined to normally modified half molecules (5'-half, nucleotides 1-36; 3'-half, nucleotides 326-75) by T4-**RNA** ligase. Using this method, we synthesized three **analogues** of yeast tRNAAla: (i) tRNAAAla which lacks base modifications in the 5'-half of the molecule; (ii) tRNAAla completely lacking base modifications in the 3'-half of the molecule; and (iii) tRNAAla completely lacking base modifications. We determined the biological activities of these **analogues**. In rat

aminoacyl-tRNA synthetase reactions, the alanine acceptance activity decreased by 52%, 79% and 85% when modified bases were absent from the 5'-half molecule, the 3'-half molecule or the total molecule, respectively. In rabbit reticulocyte lysates, alanine incorporation into proteins decreased by 3%, 57%, and 47% for tRNA **analogues** lacking modified bases in the 5'-half, and 3'-half or the entire tRNA, respectively. These results suggest that the modified nucleotides, especially in the 3'-half molecule, plays an important role in yeast tRNAAla activity.

1991

5/3,AB/154 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07589365 BIOSIS NO.: 000091118154
OLIGORIBONUCLEOTIDES SPECIFICALLY BOUND BY DNA-DEPENDENT **RNA POLYMERASE** OF ESCHERICHIA-COLI ARE NOT **ANALOGS** OF THE PROMOTER REGIONS OF THE BACTERIAL ENZYME-INTERACTING GENES
AUTHOR: SAVINKOVA L K; SOKOLENKO A A; KNORRE V L; SALGANIK R I; VEN'YAMINOVA A G; REPKOVA M N
AUTHOR ADDRESS: INST. CYTOL. GENET., SIB. DEP., ACAD. SCI. USSR, NOVOSIBIRSK, USSR.
JOURNAL: BIOPOLIM KLETKA 6 (5). 1990. 81-85. **1990**
FULL JOURNAL NAME: Biopolimery i Kletka
CODEN: BIKLE
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: Authors have verified previously advanced hypothesis that the oligoribonucleotides selectively bound by the E. coli (**T7** and T3) **RNA polymerase** mimic the promoter regions of DNA. It is shown that oligoribonucleotides homologous to the -10 region of the promoter of the nontranscribed DNA strand are not bound by E. coli **RNA polymerase** and mimic the other specific signals.
1990

5/3,AB/155 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07330755 BIOSIS NO.: 000090110657
STUDIES OF THE NUCLEOSIDE TRIPHOSPHATE BINDING SITE OF BACTERIOPHAGE **T7** DNA-DEPENDENT **RNA POLYMERASE** USING GTP **ANALOGS**
AUTHOR: AKBAROV A KH; TUNITSKAYA V L; BARANOVA L A; KHROPOV YU V; KRASIL'NIKOVA M M; KOCHETKOV S N
AUTHOR ADDRESS: V.A. ENGEL'GARDT INST. MOL. BIOL., ACAD. SCI. USSR, MOSCOW, USSR.
JOURNAL: BIOKHIMIYA 55 (5). 1990. 829-835. **1990**
FULL JOURNAL NAME: Biokhimiya
CODEN: BIOHA
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: The NTP binding site of bacteriophage **T7** DNA-dependent **RNA polymerase** was studied using GTP **analogs**. For four **analogs** the irreversible inhibition was demonstrated. The kinetic parameters for competitive (Ki) and irreversible (KI and k3) inhibition were determined. One of the **analogs**, 5'[2-hydroxy(4-iodoacetamido)benzoyl]guanosine, was shown to inactivate the enzyme rapidly due to the modification of SH-groups. Some suggestions

on the structure of the **RNA polymerase** active site have been made.

1990

5/3,AB/156 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06797964 BIOSIS NO.: 000088107403
KINETICS OF INHIBITION OF ESCHERICHIA-COLI **RNA-POLYMERASE** CATALYZED SYNTHESIS OF DINUCLEOTIDE PPPAPU BY 8 OXY-GTP AND 8 BROMO-GTP ON PROMOTER A1 OF THE BACTERIOPHAGE **T7** DELTA-111 DNA WITH A LIMITED SET OF SUBSTRATES
AUTHOR: KURYAVYI V V; USACHEVA A M; BRUSKOV V I
AUTHOR ADDRESS: INST. BIOL. PHYS., ACAD. SCI. USSR, PUSHCHINO, MOSC. OBL. 142292, USSR.
JOURNAL: MOL BIOL (MOSC) 23 (3). 1989. 822-829. **1989**
FULL JOURNAL NAME: Molekulyarnaya Biologiya (Moscow)
CODEN: MOBIB
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: Detailed analysis of the kinetics of inhibition of E. coli **RNA-polymerase**-catalyzed synthesis of dinucleotide pppApU by 8-oxy-GTP and 8-Br-GTP on promoter A1 of the bacteriophage **T7** .DELTA.111 with an incomplete set of substrates was carried out. In accordance with the mathematical models obtained, we calculated quantitative parameters of binding of these nucleotide **analogs** to the centers whose geometry is suitable for incorporation of ATP and UTP. 8-oxy-GTP and 8-Br-GTP compete with ATP for the binding center (their steady-state dissociation constant ratios are 2.1 and 2.4, respectively, whereas the constant for ATP is 0.3 mM) but, unlike ATP, they are not incorporated into the product. 8-oxy-GTP competes also with UTP (its steady-state dissociation constant ratio is 21.6, the constant for UTP is 0.03 mM). 8-Br-GTP does not interact with the binding center of UTP.

1989

5/3,AB/157 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06595513 BIOSIS NO.: 000087037675
BINDING INTERACTIONS BETWEEN YEAST TRANSFER **RNA** LIGASE AND A PRECURSOR TRANSFER **RNA** CONTAINING TWO PHOTOREACTIVE URIDINE **ANALOGUES**
AUTHOR: TANNER N K; HANNA M M; ABELSON J
AUTHOR ADDRESS: DIV. BIOL., CALIF. INST. TECHNOL., PASADENA, CALIF. 91125.
JOURNAL: BIOCHEMISTRY 27 (24). 1988. 8852-8861. **1988**
FULL JOURNAL NAME: Biochemistry
CODEN: BICHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Yeast tRNA ligase, from Saccharomyces cerevisiae, is one of the protein components that is involved in the splicing reaction of intron-containing yeast precursor tRNAs. It is an unusual protein because it has three distinct catalytic activities. It functions as a polynucleotide kinase, as a cyclic phosphodiesterase, and as an **RNA** ligase. We have studied the binding interactions between ligase and precursor tRNAs containing two photoreactive uridine **analogues**,

4-thiouridine and 5-bromouridine. When irradiated with long ultraviolet light, **RNA** containing these **analogues** can form specific covalent bonds with associated proteins. In this paper, we show that 4-thiouridine triphosphate and 5-bromouridine triphosphate were readily incorporated into a precursor tRNAPhe that was synthesized, in vitro, with bacteriophage **T7 RNA polymerase**. The **analogue**-containing precursor tRNAs were authentic substrates for the two splicing enzymes that were tested (endonuclease and ligase), and they formed specific covalent bonds with ligase when they were irradiated with long-wavelength ultraviolet light. We have determined the position of three major cross-links and one minor cross-link on precursor tRNAPhe that were located within the intron and near the 3' splice site. On the basis of these data, we present a model for the in vivo splicing reaction of yeast precursor tRNAs.

1988

5/3,AB/158 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06052321 BIOSIS NO.: 000085015470
KINETICS OF THE INHIBITION OF THE SYNTHESIS OF DINUCLEOTIDE PPPAPU CATALYZED BY ESCHERICHIA-COLI **RNA-POLYMERASE** ON PROMOTER A1 OF THE BACTERIOPHAGE **T7** DELTA-DIII DNA BY 8 OXY-ATP UNDER CONDITIONS OF COUPLED SYNTHESIS OF DINUCLEOTIDE AND TRINUCLEOTIDE AND IN THE PRESENCE OF AN INCOMPLETE SET OF SUBSTRATES
AUTHOR: KURYAVYI V V; BRUSKOV V I
AUTHOR ADDRESS: INST. BIOL. PHYS., ACAD. SCI. USSR, PUSHCHINO, USSR.
JOURNAL: MOL BIOL (MOSC) 21 (2). 1987. 462-471. **1987**
FULL JOURNAL NAME: Molekulyarnaya Biologiya (Moscow)
CODEN: MOBIB
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: A kinetic analysis of inhibition of synthesis of dinucleotide pppApU catalyzed by Escherichia coli **RNA-polymerase** on A1 promoter of the DNA from **T7** .DELTA. DIII phage mutant by 8-oxy-ATP under the conditions of the coupled synthesis of pppApU and pppApUpC and in the presence of an incomplete set of substrates, namely ATP, UTP, CTP, has been performed. It was found that 8-oxy-ATP is an unproductive **analog** of both ATP and CTP. A comparative analysis of the dissociation constants shows that 8-oxy-ATP binds at ATP center 3.3 times and at CTP center 540 times weaker than natural substrates. At the UTP center 8-oxy-ATP does not bind at all.

1987

5/3,AB/159 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05732296 BIOSIS NO.: 000084080702
EFFECT OF 8 BROMO ATP AND 8 HYDROXY-ATP ON **RNA** SYNTHESIS BY **RNA -POLYMERASE** FROM ESCHERICHIA-COLI
AUTHOR: KURYAVYI V V; BRUSKOV V I
AUTHOR ADDRESS: INST. BIOL. PHYS., ACAD. SCI. USSR, PUSHCHINO, USSR.
JOURNAL: BIOKHIMIYA 52 (1). 1987. 138-141. **1987**
FULL JOURNAL NAME: Biokhimiya
CODEN: BIOHA
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: The effect of 8-Br-ATP and 8-oxy-ATP on **RNA** synthesis on calf thymus DNA and on abortive synthesis of di- and trinucleotides on promoter AI of phage **T7** .DELTA.DIII DNA in the case of an incomplete set of substrates was studied. It was shown that the ATP **analogs** used inhibit the **RNA** and di- and trinucleotide synthesis. In all cases, 8-oxy-ATP was a more effective inhibitor than 8-Br-ATP. Both **analogs** are incapable of being the primer and they do not replace ATP in the course of abortive initiation of pppApU synthesis.
1987

5/3,AB/160 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05182138 BIOSIS NO.: 000082022759
SUBSTRATE SELECTION BY **RNA POLYMERASE** FROM ESCHERICHIA-COLI THE ROLE OF RIBOSE AND 5' TRIPHOSPHATE FRAGMENTS AND NUCLEOTIDES INTERACTION
AUTHOR: SZAFRANSKI P II; SMAGOWICZ W J; WIERZCHOWSKI K L
AUTHOR ADDRESS: INST. BIOCHEM. BIOPHYS., POL. ACAD. SCI., RAKOWIECKA 36, 02-532 POL.
JOURNAL: ACTA BIOCHIM POL 32 (4). 1985 (RECD. 1986). 329-350. **1985**
FULL JOURNAL NAME: Acta Biochimica Polonica
CODEN: ABPLA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Steady-state kinetic studies of the rifampicin-effected abortive initiation of transcription by E. coli **RNA polymerase** (EC 2.7.7.6) on the A1 **T7** phage promoter were carried out with the use of ATP, UTP and a number of their appropriately modified **analogues**. The kinetic parameters KiA, KmB, Ki and KsB characterizing the affinity of the substrates and inhibitors of the reaction to the initiation and elongation sites of the enzyme:promoter and the enzyme:promoter:nucleoside triphosphate complexes were determined therefrom. Their comparative analysis indicated that 1) the triphosphate chain of the initiating purine nucleoside triphosphate interacts with some protein acceptor groups through the .alpha.- and .beta.-phosphate residues; the phosphates are engaged in binding of nucleoside triphosphates at the elongation site in the absence of the primer nucleotide; 2) the ribose 2'-OH of the elongating nucleotide, but neither of the ribose hydroxyl groups of the initiating nucleotide, participate in substrate recognition by protein receptors; 3) either substrate, ATP or UTP, bound to the initiation complex increases by about the same factor (.gtoreq. 10) the affinity of the other to its binding site; 4) the 3'-OH of the primer nucleotide and the .gamma.-phosphate of the elongating nucleotide are involved in the synergistic interaction of the substrates; .alpha.- and .beta.-phosphates of the elongating nucleotide, bound to some protein receptors, also contribute to this process. It is postulated that the interaction of substrates is mediated through an Mg2+ ion, known to be required for binding of the substrates in the elongation site, and a minimal molecular model of a PuoTP:Mg (II): nucleoside triphosphate chelate complex in the catalytic centre of the transcription initiation open complex is proposed.

1985

5/3,AB/161 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03850793 BIOSIS NO.: 000075028866
INORGANIC PYRO PHOSPHATE **ANALOGS** IN THE PYRO PHOSPHOROLYSIS REACTION CATALYZED BY ESCHERICHIA-COLI **RNA POLYMERASE**
AUTHOR: ROZOVSKAYA T A; CHENCHIK A A; TARUSOVA N B; BIBILASHVILI R SH; KHOMUTOV R M
AUTHOR ADDRESS: INST. MOL. BIOL., ACAD. SCI. USSR, MOSCOW, USSR.
JOURNAL: MOL BIOL (MOSC) 15 (6). 1981 (RECD. 1982). 1205-1223. **1981**
FULL JOURNAL NAME: Molekulyarnaya Biologiya (Moscow)
CODEN: MOBIB
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: Processive pyrophosphorylysis of **RNA** from the ternary complex composed of **RNA polymerase**-nascent **RNA** -.DELTA.D111 phage **T7** DNA was studied in the absence of nucleoside triphosphates. A series of inorganic pyrophosphate **analogs** were investigated for their ability to sustain the reaction and to compete with PPi for the reaction. Methylenediphosphonic acid, imidodiphosphonic acid, phosphonoacetic acid, inorganic triphosphate and methylenediphosphonic acid phosphate were capable of substituting for PPi in the **RNA** degradation reaction with equal efficiency. **RNA** degradation gave rise to nucleoside monophosphates for phosphonoacetic acid, nucleoside triphosphates for PPi and inorganic triphosphate and nucleoside triphosphates **analogs** for methylenediphosphonic acid imidodiphosphonic acid and methylenediphosphonic acid phosphate. The problem of specific interaction of **RNA polymerase** with nucleoside triphosphates and PPi is discussed in terms of structural experiments for the compounds to be a potent substrate for **RNA polymerase**.

1981

5/3,AB/162 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02914989 BIOSIS NO.: 000069023105
MECHANISTIC STUDIES ON DNA DEPENDENT **RNA POLYMERASE** FROM ESCHERICHIA-COLI USING PHOSPHOROTHIOATE **ANALOGS** PART 1 INITIATION AND PYRO PHOSPHATE EXCHANGE REACTIONS
AUTHOR: YEE D; ARMSTRONG V W; ECKSTEIN F
AUTHOR ADDRESS: ABT. CHEM., MAX PLANCK-INST. EXP. MED., D-3400 GOETTINGEN, W. GER.
JOURNAL: BIOCHEMISTRY 18 (19). 1979. 4116-4120. **1979**
FULL JOURNAL NAME: Biochemistry
CODEN: BICHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The diastereomers of adenosine 5'-O-(1-thiotriphosphate) (ATP.alpha.S) and adenosine 5'-O-(2-thiotriphosphate) (ATP.beta.S) can replace ATP in the initiation reaction catalyzed by DNA dependent **RNA polymerase** from E. coli. In both cases, the Sp diastereomer is a better initiator than the Rp isomer. The diastereomers of 3'-uridyl 5'-adenosyl O,O-phosphorothioate [Up(S)A] can replace UpA in the primed initiation reaction catalyzed by **RNA polymerase**, but the Rp diastereomer is a better initiator than the Sp isomer. By using ATP or CpA as initiator and UTP.alpha.S, isomer A, as substrate, the stereochemical courses of both the initiation and primed initiation reactions, respectively, were determined with [phage] **T7** DNA template and they proceeded with inversion of configuration. Determination of the stereochemical course of the pyrophosphate exchange reaction catalyzed by **RNA polymerase** provides evidence that

this reaction is the reverse of the phosphodiester bond-forming reaction.

1979

5/3,AB/163 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02659489 BIOSIS NO.: 000067047555
PHOTO AFFINITY LABELING OF ESCHERICHIA-COLI **RNA POLYMERASE** WITH SUBSTRATE **ANALOGS** IN A TRANSCRIPTION COMPLEX
AUTHOR: SVERDLOV E D; TSAREV S A; MODYANOV N N; LIPKIN V M; GRACHEV M A; ZAICHIKOV E F; PLETNEV A G
AUTHOR ADDRESS: M.M. SHEMYAKIN INST. BIOORG. CHEM., ACAD. SCI. USSR, MOSCOW, USSR.
JOURNAL: BIOORG KHIM 4 (9). 1978. 1278-1280. **1978**
FULL JOURNAL NAME: Bioorganicheskaya Khimiya
CODEN: BIKHD
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: gamma.-Azidoanilidates of ATP and GTP are substrates of DNA-dependent **RNA polymerase** of E. coli. The **RNA** product synthesized in the presence of these **analogs** contains azidoaniline residue at the 5'-end, therefore which allows to label the corridor along which **RNA** leaves the transcribing complex. The photoaffinity labeling experiments were perfomed in the presence of 2 kinds of promoter-containing fragments, a 1000 base pair long fragment of .**GRAPHIC**. DNA containing a single promoter of 45 **RNA** and a fragment of the same length of [phage] **T7** DNA containing the early promoters A1, A2, A3. The reaction mixtures composed of a promoter-containing fragment, **RNA polymerase**, 1 of the **analogs**, a radioactive NTP [nucleoside triphosphate], and, in some cases, additional nonradioactive NTP, were irradiated by UV-light (.lambda. > 290 nm). With different substrate and template combinations varions labeling patterns were obtained involving .beta..beta.'-, .sigma.- .alpha.-subunits.

1978

5/3,AB/164 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02487961 BIOSIS NO.: 000066070518
TRANSCRIPTION OF PHAGE T-7 DNA CONTAINING MODIFIED NUCLEOTIDES BY BACTERIO PHAGE PHAGE T-7 SPECIFIC **RNA POLYMERASE**
AUTHOR: STAHL S J; CHAMBERLIN M J
AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. CALIF., BERKELEY, CALIF. 94720, USA.
JOURNAL: J BIOL CHEM 253 (14). 1978 4951-4959. **1978**
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The interaction of bacteriophage **T7** specific **RNA polymerase** with its cognate promoter sites was probed by selectively replacing bases in one **T7** promoter site with base **analogs**. Base **analogs** such as 2,6-diaminopurine or hypoxanthine, which alter residues appearing in the minor groove of the DNA helix, prevent utilization of the promoter by **T7 RNA polymerase**. These **analogs** do not affect transcription which

starts outside of the modified region. Base **analogs** that have alterations that appear in the major groove of the DNA helix, such as uracil, 5-bromouracil, 5-methylcytosine, 5-hydroxymethylcytosine and [5-HgSR]pyrimidines, do not prevent utilization of the promoter. The deoxyribonucleoside **analog** 5'-imino-5'-deoxythymidine, an alteration appearing in the deoxyribosephosphodiester backbone of the DNA helix, does not prevent promoter recognition. Haemophilus aegyptius restriction endonuclease [Hae-] III, which cleaves DNA at the sequence 5'GGCC3', does not act at sites in which the guanine residues in 1 of the 2 DNA strands were substituted with hypoxanthine. This implicates the guanine amino group in the minor groove of the DNA helix as a possible recognition point for this restriction endonuclease.

1978

5/3,AB/165 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02438574 BIOSIS NO.: 000066021117
TERMINATION OF TRANSCRIPTION BY ESCHERICHIA-COLI **RNA POLYMERASE** IN-VITRO IS AFFECTED BY RIBO NUCLEOSIDE TRI PHOSPHATE BASE **ANALOGS**
AUTHOR: NEFF N F; CHAMBERLIN M J
AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. CALIF., BERKELEY, CALIF. 94720, USA.
JOURNAL: J BIOL CHEM 253 (7). 1978 2444-2460. **1978**
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: E. coli **RNA polymerase** holoenzyme transcribes bacteriophage **T7** DNA selectively in vitro. Under normal conditions transcription is initiated predominantly at the 3 A promoter sites (near 1% on the standard genetic map) and is terminated at a site located at 20%. Transcription of DNA from the homologous bacteriophage T3 is also initiated predominantly at the left end of the genetic map, but where transcription is terminated near 20% in vivo, there is little or no termination in vitro. Ribonucleoside triphosphate base **analogs** can be incorporated into the **RNA** chain which alter the stability of the **RNA**-DNA hybrid made during transcription and substantially affect termination at the 2 termination sites in vitro. Substitution of a ribonucleoside triphosphate base **analog** (5-bromocytidine-5'-triphosphate) which stabilizes the **RNA**-DNA hybrid stimulates termination with T3 DNA. Substitution of a base **analog** (ITP) that destabilizes the **RNA**-DNA hybrid supresses termination with **T7** DNA. These 2 observations suggest that the critical step in the termination of transcription is the formation of a G-C containing **RNA**-DNA hybrid at the termination site. This model is supported by transcription studies on synthetic homopolymers (dGn) .cntdot. (dCn) and d(A-T)n. **RNA polymerase** appears to terminate and reinitiate frequently when (dG) .cntdot. (dCn) is transcribed with GTP and a stable **RNA**-DNA hybrid (rG) .cntdot. (dCn) is found. Termination occurs rarely, however, when d(A-t)n is the template or when ITP is the substrate for (dGn) .cntdot. (dCn) transcription and a free **RNA** chain is formed. It appears that the termination of transcription is stimulated when the formation of a stable **RNA**-DNA hybrid is favored over the reformation of the DNA duplex.

1978

5/3,AB/166 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02376299 BIOSIS NO.: 000065033330
ROLE OF THE INTRINSIC METAL IN **RNA POLYMERASE** EC-2.7.7.6 FROM ESCHERICHIA-COLI IN-VIVO SUBSTITUTION OF TIGHTLY BOUND ZINC WITH COBALT
AUTHOR: SPECKHARD D C; WU F Y-H; WU C-W
AUTHOR ADDRESS: DIV. BIOL. SCI., DEP. BIOPHYS., ALBERT EINSTEIN COLL. MED., NEW YORK, N.Y. 10461, USA.
JOURNAL: BIOCHEMISTRY 16 (24). 1977 5228-5233. **1977**
FULL JOURNAL NAME: Biochemistry
CODEN: BICHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: E. coli **RNA polymerase** [EC 2.7.7.6] is a metalloenzyme containing 2 g-atoms of tightly bound Zn/mol of enzyme. **RNA polymerase** from E. coli cells grown in a Zn-depleted medium supplemented with CoCl2 was prepared. The purified enzyme contains 1.8 .apprx. 2.2 g-atoms of Co/mol of enzyme with concomitant reduction in the Zn content. The Co-substituted enzyme is enzymatically as active as Zn-**RNA polymerase** on a variety of templates under standard assay conditions. These 2 enzymes are almost identical by such physical criteria as subunit composition, monomer-dimer equilibrium and pH and temperature stabilities. They differ in that Co-**RNA polymerase** exhibits a visible absorption spectrum with 2 major peaks at 584 and 703 nm. Addition of nucleoside triphosphates selectively perturbs the 584 nm peak, but the addition of a template **analog**, d(pT)10, affects both peaks. These spectral changes suggest that the tightly bound metal ions may directly or indirectly participate in binding of substrate or template to the enzyme. Biochemically, both enzymes are also very similar with respect to pH-activity profile, extrinsic metal requirements, 1,10-phenanthroline inhibition and fidelity of transcription of synthetic templates. Detailed kinetic and biochemical analyses revealed that the Co enzyme has a lower value (.apprx. 2-fold) of apparent Km for [phage] **T7** DNA under certain experimental conditions and that it is less efficient in initiating **RNA** chains at the A2 than at the A1 + A3 promoters on **T7** DNA template as compared to the Zn enzyme. This was demonstrated by studying the ratio of GTP/ATP incorporations into the 5' terminal of **RNA** products and by measuring the formation of (rI)n-resistant initiation complexes at specific promoter sites using various combinations of dinucleotides and nucleoside triphosphates. The in vitro transcription of a lac operon system by Co-**RNA polymerase** is less sensitive to c[cyclic]AMP and cAMP receptor protein than is the transcription by Zn-**RNA polymerase**. The results of comparative studies using the Co and Zn enzymes showed that the intrinsic metal of **RNA polymerase** is apparently involved in promoter recognition and specific initiation in **RNA** synthesis.

1977

5/3,AB/167 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02202235 BIOSIS NO.: 000064044754
CAULOBACTER-CRESCENTUS **RNA POLYMERASE** PURIFICATION AND CHARACTERIZATION OF HOLO ENZYME AND CORE **POLYMERASE**
AUTHOR: AMEMIYA K; WU C W; SHAPIRO L
JOURNAL: J BIOL CHEM 252 (12). 1977 4157-4165. **1977**
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract

ABSTRACT: C. crescentus **RNA polymerase** holoenzyme and core enzyme were separated by chromatography on denatured DNA-cellulose and purified by phosphocellulose chromatography. To assess the functional role of the putative C. crescentus .sigma. subunit, the enzymes were compared to Escherichia coli holoenzyme and core **RNA** polymerases, and the transcription of various DNA templates by C. crescentus core **polymerase** with E. coli was examined. C. crescentus and E. coli **RNA** polymerases differed in subunit MW and degree of immunologic cross-reactivity. Despite these differences, the E. coli .sigma. subunit partially stimulated the transcription of E. coli phage **T7** or T2 DNA templates by C. crescentus core enzyme. The presence of the .sigma. subunit influenced the specificity of nucleoside triphosphate incorporation at the 5' terminus of the **RNA** chain and the selective strand transcription of phage **T7** DNA. The C. crescentus core **polymerase** and .sigma. subunit is functionally **analogous** to the E. coli core **polymerase**-.sigma. subunit interaction. Addition of E. coli .sigma. to the C. crescentus core **polymerase**, but not to the C. crescentus holoenzyme, preferentially stimulated transcription of E. coli DNA templates. C. crescentus holoenzyme, but not core **polymerase**, formed open binary complexes with template DNA. The interaction of the .sigma. subunit with the core **polymerase** influenced the specificity of 5'-terminal ribonucleoside triphosphate incorporation and the asymmetric transcription of phage **T7** DNA template.

1977

5/3,AB/168 (Item 23 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02178086 BIOSIS NO.: 000064020599
RNA POLYMERASE EC-2.7.7.6 POTENT COMPETITIVE INHIBITION BY D RIBOSE 5 TRI PHOSPHATE AND OTHER PENTOSE POLY PHOSPHATES
AUTHOR: SYLVESTER J E; DENNIS D
JOURNAL: BIOCHEM BIOPHYS RES COMMUN 75 (3). 1977 667-673. **1977**
FULL JOURNAL NAME: Biochemical and Biophysical Research Communications
CODEN: BBRCA
RECORD TYPE: Abstract

ABSTRACT: Ribose and deoxyribose 5' pyro- and triphosphates were potent substrate competitive inhibitors for an in vitro transcription system containing calf thymus or [phage] **T7** DNA as template, and the Escherichia coli **RNA polymerase** [EC 2.7.7.6]. Each of these **analogs** gave Ki values (.apprx. 25 .mu.M) essentially the same as the Km values (.apprx. 15 .mu.M) for the substrates. The ribose and deoxyribose 5' monophosphates, ribonucleosides, deoxynucleoside mono- and triphosphates were not significant inhibitors. The enzyme apparently binds the substrate primarily through the 3'-endo ribose polyphosphate moiety.

1977

et al., 1996). Using a **T7 RNA polymerase** expression system, we produced huDHODH as a fusion protein containing an amino-terminal decahistidine tag. Escherichia coli growth and expression conditions were optimized to enhance huDHODH solubility and to permit purification of the enzyme in the absence of detergent. Soluble huDHODH, purified by a simple two-step procedure, was catalytically active, monomeric, and contained a flavin mononucleotide (FMN) cofactor in a 1:1 FMN/protein molar ratio. Kinetic analysis showed that huDHODH uses a two site ping-pong mechanism, where DHO is oxidized at one site and the second substrate, ubiquinone, is reduced at the other. This result is consistent with the mechanism proposed for bovine liver DHODH (Hines and Johnston, 1989).

5/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10471972 20017278 PMID: 10549512
RNA-protein crosslinking with photoreactive nucleotide **analogs**.
Hanna M M; Bentsen L; Lucido M; Sapre A
Department of Chemistry and Biochemistry, University of Oklahoma, Norman, USA.
Methods in molecular biology (Clifton, N.J.) (UNITED STATES) **1999**, 118 p21-33, ISSN 1064-3745 Journal Code: 9214969
Contract/Grant No.: RO1 GM47493; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

5/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10441073 99436120 PMID: 10506170
Bipartite modular structure of intrinsic, **RNA** hairpin-independent termination signal for phage **RNA** polymerases.
Kwon Y S; Kang C
Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea.
Journal of biological chemistry (UNITED STATES) Oct 8 **1999**, 274 (41) p29149-55, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The phage SP6 **RNA** and **T7 RNA** polymerases, which are closely related to each other, intrinsically stop at two signals in the Escherichia coli rrnB terminator t1 through different mechanisms. The downstream signal functioned without an **RNA** secondary structure formation, in which the signal was still active when separated from the upstream, hairpin-forming signal, and IMP incorporation enhanced its efficiency. The sequence from -15 to -1 was essential for the downstream, hairpin-independent termination (at -1). The results of SP6 transcription with heteroduplex templates and ribonucleotide **analogs** suggested that the downstream signal consists of two functionally different modules. The effects of iodo-CMP or IMP incorporation into **RNA** on termination efficiency were not sensitive to incorporation at -9 and upstream, but they were reactive to incorporation at -6 and -2, as reflected by strong iodo-rC:dG and weak rI:dC base pairing. Thus, the downstream module (from -8 approximately -6 to -1) appears to facilitate the release of **RNA**. Mismatches in the templates at -6 to +1 allowed for efficient termination, unlike those upstream of the sequence. The upstream module (from -15 to -9

```
*File 155: For updating information please see Help News155. Alert
feature enhanced with customized scheduling. See HELP ALERT.
  File   5:Biosis Previews(R)  1969-2002/Dec W2
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? s t7 and polymerase
           11928  T7
          373458  POLYMERASE
      S1    6068  T7 AND POLYMERASE
? s s1 and analog?
            6068  S1
          587026  ANALOG?
      S2     378  S1 AND ANALOG?
? s s2 and py<2000
Processing
             378  S2
        22894572  PY<2000
      S3     315  S2 AND PY<2000
? rd
...examined 50 records  (50)
...examined 50 records  (100)
...examined 50 records  (150)
...examined 50 records  (200)
...examined 50 records  (250)
...examined 50 records  (300)
...completed examining records
      S4     223  RD (unique items)
?
PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES
? ds

Set     Items   Description
S1       6068   T7 AND POLYMERASE
S2        378   S1 AND ANALOG?
S3        315   S2 AND PY<2000
S4        223   RD (unique items)
? s s4 and rna
             223  S4
          712938  RNA
      S5     168  S4 AND RNA
? t s5/3,ab/all

 5/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10841436   20395216   PMID: 10941801
  Expression and characterization of E. coli-produced soluble, functional
human dihydroorotate dehydrogenase: a potential target for
immunosuppression.
  Neidhardt E A; Punreddy S R; McLean J E; Hedstrom L; Grossman T H
  Procept, Inc., Cambridge, MA 02139, USA.
  Journal of molecular microbiology and biotechnology (ENGLAND) Aug
1999, 1 (1) p183-8, ISSN 1464-1801 Journal Code: 100892561
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Human dihydroorotate dehydrogenase (huDHODH) is essential for de novo
biosynthesis of pyrimidines and the target of two immunosuppressive drugs,
brequinar and the leflunomide metabolite A77-1726 (Chen et al., 1992; Davis
```

approximately -7) functions as a duplex. Pausing of the SP6 elongation complex at the termination site was detected when **RNA** release was suppressed by the incorporation of 5-bromo-UMP, and it was dependent on the upstream module. Results of single-round SP6 transcriptions using 3'-deoxynucleotides and immobilized templates indicated that **RNA** was not released from the elongation complexes halted at the termination site on the template variants carrying mutations in the upstream or downstream module, whereas such complexes on the wild type template were dissociated. Thus, halting or simple pausing was not sufficient for termination even when the downstream module was intact. The upstream module appears to mediate such conformation change necessary for termination.

5/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10296467 99263030 PMID: 10325415
The environment of 5S rRNA in the ribosome: cross-links to 23S rRNA from sites within helices II and III of the 5S molecule.
Osswald M; Brimacombe R
Max-Planck-Institut fur Molekulare Genetik, Ihnestrasse 73, 14195 Berlin, Germany.
Nucleic acids research (ENGLAND) Jun 1 **1999**, 27 (11) p2283-90, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Three contiguous fragments of Escherichia coli 5S rRNA were prepared by **T7** transcription from synthetic DNA templates. The central fragment, comprising residues 33-71 of the molecule, was transcribed in the presence of 4-thiouridine triphosphate together with [32P]UTP. The three transcripts were ligated together, yielding a 5S rRNA **analogue** carrying 4-thiouridine residues at positions 40, 48, 55 and 65 in helices II and III. After ligation, the 4-thiouridine residues were derivatised with p -azidophenacyl bromide. The modified 5S rRNA was reconstituted into 50S subunits and these subunits were used to prepare 70S ribosomes in the presence or absence of tRNA and mRNA. The azidophenyl groups were then photoactivated by mild irradiation at 300 nm and the products of cross-linking analysed by our standard procedures. Multiple cross-links from 5S rRNA to two distinct regions of the 23S rRNA were observed. The first region was located in helix 38 in Domain II of the 23S molecule, with cross-links at sites between nucleotides 885 and 922. The second region covered helices 81-85 in Domain V, with sites between nucleotides 2272 and 2345. Taken together with previous data, these results serve to define the arrangement of the 5S rRNA molecule relative to the 23S rRNA within the 50S subunit.

5/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10256441 99252211 PMID: 10233981
A single amino acid substitution in the phosphoprotein of respiratory syncytial virus confers thermosensitivity in a reconstituted **RNA polymerase** system.
Marriott A C; Wilson S D; Randhawa J S; Easton A J
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom.
Journal of virology (UNITED STATES) Jun **1999**, 73 (6) p5162-5, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed

The single amino acid change Gly172 to Ser in the phosphoprotein (P) of respiratory syncytial virus (RSV) has previously been shown to be responsible for the thermosensitivity and protein-negative phenotype of tsN19, a mutant of the B subgroup RSN-2 strain. This single change was inserted into the P gene of the A subgroup virus RSS-2, and the resulting phenotype was observed in a plasmid-driven reconstituted RSV **RNA polymerase** system. Expression from a genome **analogue** containing two reporter genes was thermosensitive when directed by plasmids containing the N, L, M2, and mutant P genes cloned under the control of **T7** promoters. Analysis of **RNA** synthesis showed that mutant P protein was unable to produce genome, antigenome, or mRNA at the restrictive temperature. At a semipermissive temperature, genome, antigenome, and mRNA synthesis were all reduced, 6- to 30-fold, relative to synthesis directed by a wild-type P plasmid. Binding of the mutant P protein to N protein in the absence of other viral proteins was unaffected by temperature, indicating that the lesion did not produce a large enough structural change to disrupt this binding. These data suggest that the plasmid rescue system is suitable for investigation of the role of thermosensitive mutations in RSV **polymerase** components in **RNA** synthesis.

5/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10243778 99230187 PMID: 10213599

Probing the interaction of **T7 RNA polymerase** with promoter.

Sastry S; Ross B M

Laboratory of Molecular Genetics, The Rockefeller University, New York 10021, USA. sastrys@rockvax.rockefeller.edu

Biochemistry (UNITED STATES) Apr 20 **1999**, 38 (16) p4972-81, ISSN 0006-2960 Journal Code: 0370623

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transcription is the fundamental process by which **RNA** is synthesized by **RNA** polymerases on double-stranded DNA templates. One structurally simple **RNA polymerase** is encoded by bacteriophage **T7**. **T7 RNA polymerase** is an excellent candidate for studying structural aspects of transcription, because unlike the eucaryotic and bacterial **RNA** polymerases, it is a single subunit enzyme and does not require additional factors to carry out the entire process of transcription from start to finish. An important advantage of studying transcription using this enzyme is that the high-resolution crystal structure of **T7 RNA polymerase** has been solved. However, a cocrystal structure of the **polymerase** complexed with promoter has not yet been published. Here, we have used cross-linking techniques to understand the interaction of promoter with **T7 RNA polymerase** . We constructed promoters that were substituted with the photo-cross-linkable nucleotide 5-iodo uracil at every dT in the promoter from -17 to -1. This substitution replaces the 5-methyl in dT with an iodine atom. The substituted promoters were photo-cross-linked to **T7** RNAP, and the efficiency of cross-linking was quantitated at every position. In the melting domain, the strongest contacts occurred at -3 and at -1 on the template strand while very weak cross-linking was seen at -2 and at -4 on the nontemplate strand. In the binding domain, the strongest contacts were seen at -16, -15, and -13 and at -10 on the template strand while at -17 and -14 on the nontemplate strand very weak cross-linking was observed. Cross-linking was poor in the intervening region between the binding and the melting domains. These results suggested that, in the **T7 RNA polymerase**-promoter complex, the **polymerase** molecule mainly contacts the template bases in the TATA box while the

upstream contacts are used as an anchor for DNA binding. For a systematic study designed to probe the nature of base-specific interactions in the **polymerase** -promoter complex, we used neutral salts from the Hofmeister series. In general, the order of perturbation was sulfate > citrate > acetate for anions and ammonium > magnesium > potassium for cations. Using acrylamide, a neutral hydrophobic agent to probe for nonionic contacts, we observed that at -2, -4, and -17 the contacts had a hydrophobic component, while at many other positions there was no significant effect, suggesting that the contacts in the promoter-**polymerase** complexes were predominantly ionic but at certain positions nonionic interactions also existed. To localize a specific interaction in the melting domain, we proteolyzed the cross-linked **T7** RNAP and analyzed the fragments using gel electrophoresis, mass spectrometry, and amino acid composition. High-resolution mapping indicated that amino acid residues 614-627 may be in the vicinity of the melting domain. Specifically, Y623 may contact -3 on the template strand.

5/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10172769 99141236 PMID: 9973628
Probing the environment of nascent **RNA** in Escherichia coli transcription elongation complexes utilizing a new fluorescent ribonucleotide **analog**.
Hanna M M; Yuriev E; Zhang J; Riggs D L
Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval Room 208, Norman, OK 73019-0370, USA. mhanna@chemdept.chem.ou.edu
Nucleic acids research (ENGLAND) Mar 1 **1999**, 27 (5) p1369-76, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: RO1 GM47493; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We report the synthesis and characterization of 5-thioacetamidofluorescein-uridine 5'-triphosphate (5-SF-UTP), and its application to the characterization of the environment of the nascent **RNA** during trans-cription. This **analog** specifically replaced UTP as a transcription substrate for Escherichia coli and **T7** **RNA** polymerases, and yeast **RNA** **polymerase** III. Escherichia coli transcription complexes containing **analog** incorporated at only position +21 of the **RNA** were prepared. The **RNA** was then elongated in the absence of **analog**, moving the fluorescent group further away from the enzyme active site, and the fluorescence polarization was measured. **Analog** positioned near the 3' end of the transcript exhibited significantly increased polarization relative to that of free probe, consistent with the constrained environment of the **RNA** in the DNA-**RNA** hybrid. **Analog** positioned 14 nucleotides from the 3' end exhibited significantly decreased polarization relative to that at the 3' end of the **RNA**, but only slightly above that of free **RNA** , suggesting that the probe was on the solvent-exposed surface of the **polymerase** . Molecular modeling of these **analog**-substituted RNAs produced structures consistent with the experimental data. The excellent substrate properties of this **analog** make it useful for the characterization of the environment of **RNA** not only during transcription and translation, but in any type of ribonucleoprotein complex.

5/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10115186 99096871 PMID: 9878354
Conserved core structure in the internal transcribed spacer 1 of the Schizosaccharomyces pombe precursor ribosomal **RNA**.
Lalev A I; Nazar R N
Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.
Journal of molecular biology (ENGLAND) Dec 18 **1998**, 284 (5) p1341-51, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The structure of the internal transcribed spacer 1 (ITS1) in Schizosaccharomyces pombe was examined with respect to phylogenetically conserved features in yeasts as well as the binding of transacting factors that potentially play a role in ribosomal maturation. Computer analyses and probes for nuclease protection indicate a compact, more highly organized structure than previously proposed in Saccharomyces cerevisiae, with distinct structural features which can be recognized in S. cerevisiae. These include a central extended hairpin structure as well as smaller hairpins immediately adjacent to the maturing termini. Comparisons with ITS sequences in more diverse organisms indicate that the same features also can be recognized. This is especially clear in organisms which contain very short sequences in which the putative structures are much less ambiguous. Again nuclease protection analyses in one of these, Verticillium albo-atrum, confirm a central hairpin with additional hairpins linked to the maturing termini. Protein binding and gel retardation studies with the S. pombe ITS1 further indicate that, as observed in the 3' external transcripted spacer (ETS) region, the extended hairpin is not only the site of intermediate **RNA** cleavage during rRNA processing, but also a site for specific interactions with one or more soluble factors. Taken together with other analyses on transcribed spacer regions, the present data provide evidence that the spacer regions act not only to organize the maturing terminal sequences but also may serve to organize specific soluble factors, possibly acting in a manner which is **analogous** with that of the free small nucleolar ribonucleo protein particles (snoRNPs). Copyright 1998 Academic Press

5/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10069185 99043881 PMID: 9826331
Molecular and functional characterization of Salmonella enterica serovar typhimurium poxA gene: effect on attenuation of virulence and protection.
Kaniga K; Compton M S; Curtiss R; Sundaram P
Megan Health, Inc., St. Louis, Missouri 63110, USA. kkaniga@meganhealth.com
Infection and immunity (UNITED STATES) Dec **1998**, 66 (12) p5599-606, ISSN 0019-9567 Journal Code: 0246127
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Salmonella enterica poxA mutants exhibit a pleiotropic phenotype, including reduced pyruvate oxidase activity; reduced growth rate; and hypersensitivity to the herbicide sulfometuron methyl, alpha-ketobutyrate, and amino acid **analogs** . These mutants also failed to grow in the presence of the host antimicrobial peptide, protamine. In this study, PoxA- mutants of S. enterica serovar Typhimurium (S. typhimurium) were found to be 10,000-fold attenuated in orally inoculated BALB/c mice and 1,000-fold attenuated in intraperitoneally inoculated BALB/c mice, compared to wild-type S. typhimurium UK-1. In addition, poxA mutants were found to be capable of colonizing the spleen, mesenteric lymph nodes, and Peyer's

patches; to induce strong humoral immune responses; and to protect mice against a lethal wild-type Salmonella challenge. A 2-kb DNA fragment was isolated from wild-type S. typhimurium UK-1 based on its ability to complement an isogenic poxA mutant. The nucleotide sequence of this DNA fragment revealed an open reading frame of 325 amino acids capable of encoding a polypeptide of 36.8 kDa that was confirmed in the bacteriophage **T7** expression system. Comparison of the translated sequence to the available databases indicated high homology to a family of lysyl-tRNA synthetases. Our results indicate that a mutation of poxA has an attenuating effect on Salmonella virulence. Further, poxA mutants are immunogenic and could be useful in designing live vaccines with a variety of bacterial species. To our knowledge, this is the first report on the effect of poxA mutation on bacterial virulence.

5/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09987553 98438493 PMID: 9765248
Selective inhibition of HIV-1 reverse transcriptase by an antiviral inhibitor, (R)-9-(2-Phosphonylmethoxypropyl)adenine.
Suo Z; Johnson K A
Department of Biochemistry and Molecular Biology, the Pennsylvania State University, University Park, Pennsylvania 16802, USA.
Journal of biological chemistry (UNITED STATES) Oct 16 **1998**, 273 (42) p27250-8, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM44613; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
R)-9-(2-Phosphonylmethoxypropyl)adenine (PMPA) is an acyclic nucleoside phosphonate that has been shown to be effective in the treatment of AIDS although it has a shorter separation between the adenine and phosphorus than dideoxy-AMP and dAMP. By using pre-steady state kinetic methods, we examined the incorporation of the diphosphate of PMPA, 2',3'-dideoxyadenosine 5'-triphosphate (ddATP), and dATP catalyzed by wild-type human immunodeficiency virus type 1 (HIV-1) reverse transcriptase, an exonuclease-deficient **T7** DNA **polymerase** (**T7** exo-), and wild-type rat DNA **polymerase** beta in order to evaluate the selectivity of PMPA as an antiviral inhibitor. With a DNA/DNA or DNA/**RNA** 22/43-mer duplex, the diphosphate of PMPA (PMPApp) is as effective as ddATP in reactions catalyzed by HIV-1 reverse transcriptase in that both **analogs** have similar substrate specificity constants (kp/Kd) which are only 5-fold lower than dATP. In contrast, PMPApp is a much weaker inhibitor of the reaction catalyzed by **T7** exo- (with the DNA/DNA 22/43-mer duplex) in that PMPApp has a 5 x 10(-4)-fold lower kp/Kd than ddATP and dATP. The lower kp/Kd of PMPApp is due to a 1000-2000-fold lower incorporation rate (kp) and a 35-45-fold lower binding constant (Kd). Similarly, PMPApp is 800-fold less inhibitory toward **polymerase** beta with the DNA/DNA 22/43-mer duplex, whereas in studies with a single nucleotide gapped DNA (22-20/43-mer) PMPApp is 13-fold less inhibitory than ddATP. Although parallel studies will need to be performed using appropriate human polymerases, these results begin to define the mechanistic basis for the reported lower toxicity of PMPA in the treatment of AIDS.

5/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09979611 98409449 PMID: 9737873
Identifying **RNA** minor groove tertiary contacts by nucleotide **analogue** interference mapping with N2-methylguanosine.

Ortoleva-Donnelly L; Kronman M; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
Biochemistry (UNITED STATES) Sep 15 **1998**, 37 (37) p12933-42, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Nucleotide **analogue** interference mapping (NAIM) is a general biochemical method that rapidly identifies the chemical groups important for **RNA** function. In principle, NAIM can be extended to any nucleotide that can be incorporated into an in vitro transcript by an **RNA polymerase** . Here we report the synthesis of 5'-O-(1-thio)-N2-methylguanosine triphosphate (m2GalphaS) and its incorporation into two reverse splicing forms of the Tetrahymena group I intron using a mutant form of **T7 RNA polymerase**. This **analogue** replaces one proton of the N2 exocyclic amine with a methyl group, but is as stable as guanosine (G) for secondary structure formation. We have identified three sites of m2GalphaS interference within the Tetrahymena intron: G22, G212, and G303. All three of these guanosine residues are known to utilize their exocyclic amino groups to participate in tertiary hydrogen bonds within the ribozyme structure. Unlike the interference pattern with the phosphorothioate of inosine (IalphaS, an **analogue** that deletes the N2 amine of G), m2GalphaS substitution did not cause interference at positions attributable to secondary structural stability effects. Given that the **RNA** minor groove is likely to be widely used for helix packing, m2GalphaS provides an especially valuable reagent to identify **RNA** minor groove tertiary contacts in less well-characterized RNAs.

5/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09975416 98418505 PMID: 9747733
Sequence analysis of a functional **polymerase** (L) gene of bovine respiratory syncytial virus: determination of minimal trans-acting requirements for **RNA** replication.
Yunus A S; Collins P L; Samal S K
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park 20742, USA.
Journal of general virology (ENGLAND) Sep **1998**, 79 (Pt 9) p2231-8, ISSN 0022-1317 Journal Code: 0077340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The complete nucleotide sequence of a functional clone of the large **polymerase** (L) gene of bovine respiratory syncytial virus (BRSV) strain A51908 was determined by analysis of cloned cDNAs obtained from genomic and mRNAs. The BRSV L gene is 6573 nt in length and the derived polypeptide has 2162 aa. Alignment of the sequences of the BRSV L gene, and its encoded protein, with sequences of the L gene and protein of human respiratory syncytial virus strain A2 showed 77% identity at the nucleotide level and 84% identity at the amino acid level. By comparison, the L gene and protein of avian pneumovirus showed only 50% identity at the nucleotide level and 64% identity at the amino acid level. A minigenome was constructed to encode a BRSV vRNA **analogue** containing the gene for chloramphenicol acetyltransferase (CAT) under the control of putative BRSV transcription motifs and flanked by the BRSV genomic termini. Transfection of plasmids encoding the BRSV minigenome, nucleocapsid protein (N), phosphoprotein (P) and L protein, each under the control of **T7** promoter, into cells infected with a vaccinia virus recombinant expressing

the **T7 RNA polymerase** gave rise to CAT activity and progeny with the minigenome. This result indicates that the N, P and L proteins are necessary and sufficient for transcription and replication of the BRSV minigenome and are functional. Further, inclusion of small amounts of the M2 protein along with the N, P and L proteins greatly augmented minigenome transcription.

5/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09949071 98377453 PMID: 9711574
Functional expression of human P-glycoprotein from plasmids using vaccinia virus-bacteriophage **T7 RNA polymerase** system.
Hrycyna C A; Ramachandra M; Pastan I; Gottesman M M
Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.
Methods in enzymology (UNITED STATES) **1998**, 292 p456-73, ISSN 0076-6879 Journal Code: 0212271
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

5/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09903261 98337907 PMID: 9671810
Intracellular **RNA** cleavage by the hairpin ribozyme.
Seyhan A A; Amaral J; Burke J M
Markey Center for Molecular Genetics, Department of Microbiology and Molecular Genetics, 306 Stafford Hall, The University of Vermont, Burlington, VT 05405, USA.
Nucleic acids research (ENGLAND) Aug 1 **1998**, 26 (15) p3494-504 , ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Studies involving ribozyme-directed inactivation of targeted **RNA** molecules have met with mixed success, making clear the importance of methods to measure and optimize ribozyme activity within cells. The interpretation of biochemical assays for determining ribozyme activity in the cellular environment have been complicated by recent results indicating that hammerhead and hairpin ribozymes can cleave **RNA** following cellular lysis. Here, we report the results of experiments in which the catalytic activity of hairpin ribozymes is monitored following expression in mammalian cells, and in which post-lysis cleavage is rigorously excluded through a series of biochemical and genetic controls. Following transient transfection, self-processing transcripts containing active and inactive hairpin ribozymes together with cleavable and non-cleavable substrates were generated within the cytoplasm of mouse OST7-1 cells using **T7 RNA polymerase**. Unprocessed **RNA** and products ofintracellular cleavage were detected and analyzed using a primer-extension assay. Ribozyme-containing transcripts accumulated to a level of 4 x 10(4) copies per cell, and self-processing proceeded to an extent of >75% within cells. Cellular **RNA** processing was blocked by mutations within the ribozyme (G8A, G21U) or substrate (DeltaA-1) that, in vitro , eliminate cleavage without affecting substrate binding. In addition to self-processing activity, trans -cleavage reactions were supported by the ribozyme-containing product of the self-processing reaction, and by the ribozyme linked to the non-cleavable substrate **analog**. Ribozyme activity was present in extracts of cells expressing constructs with active

ribozyme domains. These results provide direct biochemical evidence for the catalytic activity of the hairpin ribozyme in a cellular environment, and indicate that self-processing ribozyme transcripts may be well suited for cellular **RNA**-inactivation experiments.

5/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09799714 98241249 PMID: 9582093
The chemical basis of adenosine conservation throughout the Tetrahymena ribozyme.
Ortoleva-Donnelly L; Szewczak A A; Gutell R R; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
RNA (New York, N.Y.) (UNITED STATES) May **1998**, 4 (5) p498-519, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM48207; GM; NIGMS; GM54839; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adenosines are present at a disproportionately high frequency within several **RNA** structural motifs. To explore the importance of individual adenosine functional groups for group I intron activity, we performed Nucleotide **Analog** Interference Mapping (NAIM) with a collection of adenosine **analogues**. This paper reports the synthesis, transcriptional incorporation, and the observed interference pattern throughout the Tetrahymena group I intron for eight adenosine derivatives tagged with an alpha-phosphorothioate linkage for use in NAIM. All of the **analogues** were accurately incorporated into the transcript as an A. The sites that interfere with the 3'-exon ligation reaction of the Tetrahymena intron are coincident with the sites of phylogenetic conservation, yet the interference patterns for each **analogue** are different. These interference data provide several biochemical constraints that improve our understanding of the Tetrahymena ribozyme structure. For example, the data support an essential A-platform within the J6/6a region, major groove packing of the P3 and P7 helices, minor groove packing of the P3 and J4/5 helices, and an axial model for binding of the guanosine cofactor. The data also identify several essential functional groups within a highly conserved single-stranded region in the core of the intron (J8/7). At four sites in the intron, interference was observed with 2'-fluoro A, but not with 2'-deoxy A. Based upon comparison with the P4-P6 crystal structure, this may provide a biochemical signature for nucleotide positions where the ribose sugar adopts an essential C2'-endo conformation. In other cases where there is interference with 2'-deoxy A, the presence or absence of 2'-fluoro A interference helps to establish whether the 2'-OH acts as a hydrogen bond donor or acceptor. Mapping of the Tetrahymena intron establishes a basis set of information that will allow these reagents to be used with confidence in systems that are less well understood.

5/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09787825 98213737 PMID: 9547267
Synthesis and **RNA polymerase** incorporation of the degenerate ribonucleotide **analogue** rPTP.
Moriyama K; Negishi K; Briggs M S; Smith C L; Hill F; Churcher M J; Brown D M; Loakes D
Gene Research Centre, Okayama University, Tsushima, Okayama 700, Japan, Nycomed Amersham plc, Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire HP7 9LL, UK.

Nucleic acids research (ENGLAND) May 1 **1998**, 26 (9) p2105-11,
ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The synthesis and enzymatic incorporation into **RNA** of the hydrogen bond degenerate nucleoside **analogue** 6-(beta-d-ribofuranosyl)-3, 4-dihydro-8H-pyrimido[4,5-c]-[1,2]oxazin-7-one (P) is described. The 5'-triphosphate of this **analogue** is readily incorporated by T3, **T7** and SP6 **RNA** polymerases into **RNA** transcripts, being best incorporated in place of UTP, but also in place of CTP. When all the uridine residues in an HIV-1 TAR **RNA** transcript are replaced by P the transcript has similar characteristics to the wild-type TAR **RNA**, as demonstrated by similar melting temperatures and CD spectra. The P-substituted TAR transcript binds to the Tat peptide ADP-1 with only 4-fold lowered efficiency compared with wild-type TAR.

5/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09740162 98169155 PMID: 9510335
Enzymatic incorporation of 2'-thio-CTP into the HDV ribozyme.
Raines K; Gottlieb P A
Department of Biological Science, SUNY at Buffalo, New York 14260, USA.
RNA (New York, N.Y.) (UNITED STATES) Mar **1998**, 4 (3) p340-5,
ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM52033; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have synthesized the **analogue** 2'-deoxy-2'-thio-CTP (CTP-SH) and tested its ability to support **RNA** transcription in place of CTP. The modified nucleotide in a transcription reaction and in the absence of CTP generated the appropriately sized fragment when a mutant **T7 polymerase** (Y639F) was used. Wild-type **polymerase** was unable to generate **RNA** under the same conditions. Transcription was optimal around pH 7.5 and was dependent upon CTP-SH concentration. Transcripts containing the **analogue** were efficiently isolated using a thiol-activated sepharose column. Insertion of CTP-SH into the HDV ribozyme, replacing all cytidine residues with 2'-thiocytidine, appears to inhibit self-cleaving activity, even in the presence of manganese. The ability to introduce the CTP-SH **analogue** enzymatically into **RNA** opens the way for new structure-function studies where the 2'-hydroxyl can be efficiently replaced by a thiol group.

5/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09676237 98097405 PMID: 9436905
Structural requirements for enzymatic formation of threonylcarbamoyladenosine (t6A) in tRNA: an in vivo study with Xenopus laevis oocytes.
Morin A; Auxilien S; Senger B; Tewari R; Grosjean H
Laboratoire d'Enzymologie et Biochimie Structurales, C.N.R.S., Gifsur-Yvette, France. morin@lebs.cnrs-gif.fr
RNA (New York, N.Y.) (UNITED STATES) Jan **1998**, 4 (1) p24-37,
ISSN 1355-8382 Journal Code: 9509184
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed

We have investigated the specificity of the eukaryotic enzymatic machinery that transforms adenosine at position 37 (3' adjacent to anticodon) of several tRNAs into threonylcarbamoyladenosine (t6A37). To this end, 28 variants of yeast initiator tRNAMet and yeast tRNAVal, devoid of modified nucleotide, were produced by in vitro transcription with **T7 polymerase** of the corresponding synthetic tRNA genes and microinjected into the cytoplasm of Xenopus laevis oocytes. Threonylcarbamoyl incorporation was analyzed in tRNA transcripts mutated in the anticodon loop by substitution, deletion, or Insertion of nucleotides, or in the overall 3D structure of the tRNA by altering critical tertiary interactions. Specifically, we tested the effects of altering ribonucleotides in the anticodon loop, changes of the loop size, perturbations of the overall tRNA 3D structure due to mutations disruptive of the tertiary base pairs, and truncated tRNAs. The results indicate that, in addition to the targeted A37, only U36 was absolutely required. However, A38 in the anticodon loop considerably facilitates the quantitative conversion of A37 into t6A37 catalyzed by the enzymes present in X. laevis. The anticodon positions 34 and 35 were absolutely "neutral" and can accept any of the four canonical nucleotides A, U, C, or G. The anticodon loop size may vary from six to eight nucleotides, and the anticodon stem may have one mismatch pair of the type AxC or GxU at location 30-40 without affecting the efficiency of t6A37 formation and still t6A37 is efficiently formed. Although threonylcarbamoylation of A37 occurred with tRNA having limited perturbations of 3D structure, the overall L-shaped architecture of the tRNA substrate was required for efficient enzymatic conversion of A37 to t6A37. These results favor the idea that unique enzymatic machinery located in the oocyte cytoplasm catalyzes the formation of t6A37 in all U36A37-containing tRNAs (anticodon NNU). Microinjection of the yeast tRNAMeti into the cytoplasm of X. laevis oocytes also revealed the enzymatic activities for several other nucleotide modifications, respectively m1Gg, m2G10, m(2)2G26, m7G46, D47, m5C48/49, and m1A58.

5/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09639618 98060914 PMID: 9396824

Single substitutions of phosphorothioates in the HDV ribozyme G73 define regions necessary for optimal self-cleaving activity.

Prabhu N S; Dinter-Gottlieb G; Gottlieb P A

Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104, USA.

Nucleic acids research (ENGLAND) Dec 15 **1997**, 25 (24) p5119-24
, ISSN 0305-1048 Journal Code: 0411011

Contract/Grant No.: AI31821; AI; NIAID; GM52033; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Phosphorothioate (NTPalphaS) **analogues** were incorporated into the HDV genomic ribozyme by transcription with **T7 polymerase**. The introduction of a sulfur in place of the pro-Rp oxygen at the phosphate 5'to positions A64, A63, A43, U27, G62, C61, C44, C41, C22and C21appeared to inhibit self-cleavage activity of the G73 genomic ribozyme. Except for position C22, elevated levels of Mg2+rescued the reaction to various extents. When the sites were identified in the **RNA** sequence, they were clustered in three distinct regions that, in the secondary structure models, are predicted to be primarily single-stranded. Two of these regions have been proposed to form extensive interactions that are thought to involve a homopurine base pair. The third region is thought to be directly associated with assembly of the cleavage site.

5/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09625801 98037710 PMID: 9371659
Rescue of synthetic minireplicons establishes the absence of the NS1 and NS2 genes from avian pneumovirus.
Randhawa J S; Marriott A C; Pringle C R; Easton A J
Department of Biological Sciences, University of Warwick, Coventry, United Kingdom.
Journal of virology (UNITED STATES) Dec **1997**, 71 (12) p9849-54
, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have determined the nucleotide sequences of the regions 3' and 5' proximal to the avian pneumovirus (APV) N and L genes, respectively. These sequences were used in the construction of a synthetic minireplicon construct in which the chloramphenicol acetyltransferase (CAT) reporter gene was flanked at its 3' end with the APV leader together with the APV N gene start signal and at its 5' end with the APV L gene end signal and the genome trailer region. The ability of **T7 RNA polymerase** runoff transcripts to direct the replication and expression of the CAT reporter gene in APV-infected cells demonstrated the ability of the putative leader and trailer regions to direct genome replication and gene expression. Furthermore, this confirms the absence of the NS1 and NS2 gene **analogs** within the APV genome. We were able to detect the expression of CAT protein from cells that had been infected with supernatants from the initially infected and transfected cells. These results have identified the cis-acting sequences of APV responsible for viral replication, gene expression, and packaging into virus-like particles.

5/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09603005 98022970 PMID: 9356355
The CM2 protein of influenza C virus is an oligomeric integral membrane glycoprotein structurally **analogous** to influenza A virus M2 and influenza B virus NB proteins.
Pekosz A; Lamb R A
Howard Hughes Medical Institute, Northwestern University, Evanston, Illinois 60208-3500, USA.
Virology (UNITED STATES) Oct 27 **1997**, 237 (2) p439-51, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: R37 AI-20201; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have undertaken a characterization of the CM2 protein of influenza C virus. The CM2 coding region of **RNA** segment 6 (nucleotides 731-1147) was cloned from two strains of influenza C virus and expressed using the vaccinia virus-bacteriophage **T7 RNA polymerase** (vac-**T7**) system. An antiserum raised to a C-terminal peptide in the CM2 open reading frame recognized the CM2 protein in influenza C virus-infected cells and after vac-**T7** expression of the CM2 open reading frame. CM2 is posttranslationally modified by addition of high-mannose carbohydrate chains (Mr approximately 18 kDa) and by further addition of polylactosaminoglycans (Mr approximately 21-35 kDa). The available data indicate that CM2 has a cleavable signal peptide at the N-terminus of the protein. Site-directed mutagenesis eliminated the single potential N-linked carbohydrate attachment site on CM2 and indicated that the protein has an NoutCin orientation in membranes. Nonreducing SDS-PAGE indicated that the

protein was expressed as disulfide-linked dimers and tetramers. Cell surface biotinylation and indirect immunofluorescence showed the protein to be expressed at the cell surface. Elimination of the N-linked carbohydrate attachment site and addition of a C-terminal HA epitope tag did not adversely affect surface expression of CM2. The NoutCin membrane orientation of CM2, the size of the ectodomain and cytoplasmic tail of CM2, and its ability to form disulfide-linked oligomers are reminiscent of the structural properties of influenza A virus M2 and influenza B virus NB proteins. Copyright 1997 Academic Press.

5/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09557106 97473529 PMID: 9332383
An epitope tagged mammalian/prokaryotic expression vector with positive selection of cloned inserts.
Schneider S; Georgiev O; Buchert M; Adams M T; Moelling K; Hovens C M
Institut fur Medizinische Virologie, Universitat Zurich, Switzerland.
Gene (NETHERLANDS) Sep 15 **1997**, 197 (1-2) p337-41, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A dual eukaryotic/prokaryotic expression vector has been developed which combines the features of positive selection for cloned inserts along with the production of an epitope-tagged cDNA insert by transient transfection in mammalian cells as well as high level induced expression in E. coli cells harbouring **T7 RNA polymerase**. This vector, pZilch, has two MCSs flanking a mutant E. coli phenylalanyl-tRNA synthetase gene, pheS, which when expressed in combination with the phenylalanine **analog** p-CI-Phe, results in termination of host cell protein synthesis. Cloning of inserts using unique sites in the flanking MCS regions results in loss of the pZilch pheS allele and hence permits growth of colonies harbouring recombinants on p-Cl-Phe plates. Additional features of the vector include an optimal Kozak consensus sequence for high level eukaryotic cell expression and an efficient prokaryotic translation initiation site in frame and downstream from the eukaryotic initiation site. Recombinant proteins can be produced with an N-terminal FLAG epitope which can be removed via a specific protease cleavage site. Flanking **T7** and SP6 **RNA polymerase** promoter sites permit in vitro transcription and translation of cloned inserts. A derivative of the vector has also been constructed enabling nuclear accumulation of the tagged proteins via an SV40 nuclear localisation signal upstream of the 5' MCS.

5/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09534383 97436528 PMID: 9292501
Photoaffinity labeling of 30S-subunit proteins S7 and S11 by 4-thiouridine-substituted tRNA(Phe) situated at the P site of Escherichia coli ribosomes.
Rosen K V; Zimmerman R A
Department of Biochemistry & Molecular Biology, University of Massachusetts, Amherst 01003-4505, USA.
RNA (New York, N.Y.) (UNITED STATES) Sep **1997**, 3 (9) p1028-36, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM22807; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

4-Thiouridine, a photoreactive **analogue** of uridine, was randomly incorporated into yeast tRNA(Phe) precursor molecules by transcription with **T7 RNA polymerase** and the resulting transcripts were converted into mature tRNA(Phe) by treatment with RNase P **RNA**. The photoreactive tRNA(Phe) was aminoacylated and bound to the P site of Escherichia coli 70S ribosomes in the presence of a poly(U) template. Irradiation of the complexes with light of 300 nm resulted in the covalent crosslinking of nt U20 in the D loop of the tRNA to protein S11 of the 30S ribosomal subunit, whereas nt U33 in the anticodon loop crosslinked to 30S-subunit protein S7. These results allowed us to map the D loop of P site-bound tRNA to the platform of the 30S ribosomal subunit and provided additional information about contacts between protein S7 and the anticodon loop in the cleft between the platform and the subunit head.

5/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09497747 97392806 PMID: 9245591
Bioincorporation of telluromethionine into proteins: a promising new approach for X-ray structure analysis of proteins.
Budisa N; Karnbrock W; Steinbacher S; Humm A; Prade L; Neuefeind T; Moroder L; Huber R
Max-Planck Institut fur Biochemie, Martinsried, Germany.
Journal of molecular biology (ENGLAND) Jul 25 **1997**, 270 (4) p616-23, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A simple and efficient method for the specific and quantitative replacement of the naturally occurring amino acid methionine by its isosteric **analogue** telluromethionine in the expression of recombinant proteins has been developed. The method requires a controlable and competitive expression system like the bacteriophage **T7 polymerase** /promoter in a methionine-auxotrophic host. Using methionine-auxotrophic Escherichia coli strains, incorporation of telluromethionine at high yields has been achieved for human recombinant annexin V, human mitochondrial transamidase, Arabidopsis glutathione-S-transferase and the N-terminal domain of Salmonella tailspike adhesion protein as confirmed by amino acid, mass-spectrometric and X-ray analyses. Expressed and purified telluromethionine-proteins and native proteins were found to crystallise isomorphously. In terms of efficient bio-expression, isomorphism of crystals and relative abundance of methionine residues, the production of telluromethionine-proteins as heavy-atom derivatives offers a valid and general approach in X-ray analysis by the method of multiple isomorphous replacement.

5/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09477031 97378267 PMID: 9234948
Minimum protein requirements for transcription and **RNA** replication of a minigenome of human parainfluenza virus type 3 and evaluation of the rule of six.
Durbin A P; Siew J W; Murphy B R; Collins P L
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892-0720, USA.
Virology (UNITED STATES) Jul 21 **1997**, 234 (1) p74-83, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
A reconstituted transcription and **RNA** replication system for human parainfluenza virus type 3 (HPIV3) was developed using components expressed intracellularly from transfected plasmids driven by **T7 RNA polymerase** supplied by a vaccinia virus recombinant. The system is based on a negative-sense **analog** of HPIV3 genomic **RNA** in which the viral genes were deleted and replaced with that encoding bacterial chloramphenicol acetyl transferase (CAT). The N, P, and L proteins expressed from cotransfected plasmids were necessary and sufficient to direct efficient transcription and **RNA** replication. Transcription yielded subgenomic polyadenylated mRNA, which was isolated by oligo(dT) chromatography. **RNA** replication yielded a mini-antigenome and progeny minigenome, which were shown to be encapsidated based on resistance to digestion with micrococcal nuclease. A panel of cDNAs was constructed to encode minigenomes which differed in length by single-nucleotide increments. Transcription and **RNA** replication in the reconstituted system were most efficient for the minigenome whose length was an even multiple of six. Both **RNA** replication and transcription appeared to be governed by the rule. However, minigenomes whose lengths were one nucleotide greater than or less than an even multiple of six also were very active, especially in **RNA** replication, indicating that the rule was not absolute.

5/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09410004 97309491 PMID: 9166906
Single molecular assay of individual ATP turnover by a myosin-GFP fusion protein expressed in vitro.
Iwane A H; Funatsu T; Harada Y; Tokunaga M; Ohara O; Morimoto S; Yanagida T
Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Japan. iwane@bpe.es.osaka-u.ac.jp
FEBS letters (NETHERLANDS) Apr 28 **1997**, 407 (2) p235-8, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Fusion proteins of a truncated mutant of myosin subfragment-1 (S1dC) and green fluorescent protein (GFP) were expressed in vitro by **T7 RNA polymerase** and rabbit reticulocyte lysate. Single S1dC-GFP fusion proteins were clearly seen and their individual ATP turnovers were directly monitored using low background total internal reflection fluorescence microscopy (LBTIRFM), recently developed by our laboratory. LBTIRFM using GFP as a fluorescent tag allowed us to assay functions of single protein molecules expressed in vitro. Thus, the results suggested that this method may be particularly useful to analyze functions of proteins that cannot be produced in an active form and/or in large quantities in conventional heterologous expression systems.

5/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09314537 97233196 PMID: 9078386
Initiation of Sendai virus multiplication from transfected cDNA or **RNA** with negative or positive sense.
Kato A; Sakai Y; Shioda T; Kondo T; Nakanishi M; Nagai Y
Department of Viral Infection, University of Tokyo, Japan.
Genes to cells : devoted to molecular & cellular mechanisms (ENGLAND) Jun **1996**, 1 (6) p569-79, ISSN 1356-9597 Journal Code: 9607379
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: The mononegavirus superfamily (Mononegavirales) comprises three families, Rhabdoviridae, Paramyxoviridae and Filoviridae. These viruses possess a single stranded negative sense **RNA** as the genome. Recent success in the recovery of infectious virus from a transfected cDNA of mononegaviruses including Sendai virus, a prototypic paramyxovirus, is opening the possibility of their genetic engineering. However, infectious viruses have been recovered only by initiating the infectious cycle with cDNA directing the synthesis of antigenomic positive sense (+)**RNA**. Starting with genomic negative sense (-)**RNA** has been unsuccessful. Furthermore, the recovery efficiency has often been extremely low. RESULTS: We describe here an **analogous** system that allows recovery of Sendai virus at a high rate, from cells in which the transfected cDNA and plasmids to support the synthesis of viral nucleocapsid protein and **RNA** polymerases are coexpressed by vaccinia virus-driven bacteriophage **T7 polymerase** . Our system was able to recover the virus from cDNA directing not only (+)**RNA** but also (-)**RNA**. Moreover, using this system, we succeeded in recovery of the virus by transfection of in vitro synthesized (+)**RNA** or (-)**RNA** . This improved virus recovery appeared to be accomplished by supplying the supporting plasmids at an optimal ratio and by minimizing the cytopathic effect of the vaccinia virus by specific inhibitors. In addition, it was probably critical that our cDNAs were constructed to generate viral authentic RNAs without adding **T7** promoter-specific nucleotides to the 5' ends. An immediate application of the system was demonstrated by the creation of a candidate vaccine strain with a predetermined attenuating mutation in the cleavage-activation site of the viral fusion glycoprotein. CONCLUSION: We have established methods which greatly improve the recovery of Sendai virus from cDNA. There is essentially no absolute obstacle to recovery of the virus from the (-)**RNA** template. Even the complete full length **RNA** chain in the naked form appears to be properly encapsidated to become a functional template.

5/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09294948 97207059 PMID: 9054552
Inhibition of bacteriophage **T7 RNA polymerase** in vitro transcription by DNA-binding pyrrolo[2,1-c][1,4]benzodiazepines.
Puvvada M S; Forrow S A; Hartley J A; Stephenson P; Gibson I; Jenkins T C ; Thurston D E
Gene Targeted Drug Design Research Group, School of Pharmacy and Biomedical Science, University of Portsmouth, U.K.
Biochemistry (UNITED STATES) Mar 4 **1997**, 36 (9) p2478-84, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The interactions of several pyrrolo[2, 1-c][1,4]benzodiazepine (PBD) antitumor antibiotics with linearized plasmid pGEM-2-N-ras DNA have been analyzed by quantitative in vitro transcription (QIVT) and in vitro transcription footprinting (IVTF) methods. A concentration-dependent inhibitory effect of the PBDs on transcription is observed using both techniques. The rank order for overall inhibition of transcription by the QIVT method is found to be: sibiromycin > tomaymycin > anthramycin > DC-81 > neothramycin, whereas the IVTF experiments show a different ranking: sibiromycin > anthramycin > neothramycin > tomaymycin. In addition, stimulation of transcription was observed at low PBD concentrations in both the QIVT and IVTF experiments. These results demonstrate unequivocally that the formation of PBD-DNA adducts at AGA-5' base sequences on the

transcribed strand results in transcription blockage for all PBDs examined. Furthermore, the sequence of flanking base pairs appears to influence the degree of blocking, with the sequences ACAGAAA-5', AAAGATG-5', AGAGATA-5', and CAAGAAC-5' providing the most pronounced blocks for all PBDs studied in this system. Neothramycin and tomaymycin cause additional blocks at some GGA-5' and TGA-5' sequences. Parallel MPE-Fe(II) footprinting studies have revealed PBD binding sites on both the transcribing and nontranscribing strands, although all transcription blocks determined from the IVTF assays are due to drug bound on the transcribing DNA template strand.

5/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09276454 97166229 PMID: 9013628
Cross-linking of DNA-binding proteins to DNA with psoralen and psoralen furan-side monoadducts. Comparison of action spectra with DNA-DNA cross-linking.
Sastry S S; Ross B M; P'arraga A
Laboratory of Molecular Genetics, and the Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021, USA. sastrys@rockvax.rockefeller.edu
Journal of biological chemistry (UNITED STATES) Feb 7 **1997**, 272 (6) p3715-23, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have developed a novel photocross-linking technique using free 8-methoxypsoralen and DNA furan-side monoadducts plus long wave ultraviolet light (UVA). Both sequence-specific (Max) and nonspecific (RecA and **T7 RNA polymerase**) DNA-binding proteins were cross-linked. The macroscopic equilibrium binding constant (approximately 10(9) M-1) and DNase I footprinting indicated that binding of Max to its cognate sequence (E-box) was unimpaired by 8-methoxypsoralen and that cross-linking occurred in normal complexes. RecA protein and **T7 RNA polymerase** were cross-linked to a 12-mer DNA furan-side monoadduct with UVA. Cross-link yields were directly proportional to the UVA dose. Cross-links were stable to 8 M urea, 1-10% SDS, commonly used alcohols, and mild acids (5% trichloroacetic acid). The DNA in cross-links was reversed with 254 nm UV (photoreversal) or with hot base (base-catalyzed reversal), consistent with (2 + 2) cycloaddition via the 4',5'-furan of the psoralen. Comparative action spectra for DNA-DNA cross-linking and DNA-protein cross-linking revealed that the latter occurred maximally at 300 nm, while the former occurred maximally at 320 nm. This 20-nm blue shift suggested a higher potential energy surface for an excited psoralen participating in protein-DNA cross-linking as compared with DNA-DNA cross-linking. As with DNA-DNA cross-linking, DNA-protein cross-linking is a two-photon process. Absorption of the first photon formed a 4',5'-adduct with DNA, which then absorbed a second photon, leading to cross-linking to protein. Based on the action spectra and the known excited states of psoralen, it is suggested that the triplet n,pi* transition localized in the C-2=O of psoralen may be involved in protein-psoralen photoreactions.

5/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09268843 97151114 PMID: 8995650
Rescue of rinderpest virus from cloned cDNA.
Baron M D; Barrett T
Institute for Animal Health, Pirbright, Surrey, United Kingdom. michael.baron@bbsrc.ac.uk

Journal of virology (UNITED STATES) Feb 1997, 71 (2) p1265-71, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Rinderpest virus is a morbillivirus and is the causative agent of a widespread and important disease of cattle. The viral genome is a single strand of **RNA** in the negative sense. We have constructed plasmids containing cDNA copies of the 5' and 3' termini of the virus separated by a reporter gene and have shown that antigenome-sense **RNA** transcripts of these model genomes can be replicated, transcribed, and packaged by helper virus, both rinderpest virus and the related measles virus. Further, these genome **analogs** can be replicated and transcribed by viral proteins expressed from cDNA clones by using a recombinant vaccinia virus expressing **T7 RNA polymerase** (MVA-**T7**). Using this latter system, we have rescued live rinderpest virus from a full-length cDNA copy of the genome of the RBOK vaccine strain. The recombinant virus appears to grow in tissue culture identically to the original virus.

5/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09267220 97150984 PMID: 8995520
A model for the mechanism of **polymerase** translocation.
Guajardo R; Sousa R
Department of Biochemistry, University of Texas Health Science Center, San Antonio 78284-7760, USA.
Journal of molecular biology (ENGLAND) Jan 10 **1997**, 265 (1) p8-19, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM-52522; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A general mechanism for **polymerase** translocation is elaborated. The central feature of this mechanism is that a rapid translocational equilibrium is established after each cycle of nucleoside monophosphate incorporation such that the **polymerase** distributes itself by diffusional sliding between all accessible positions on the template with relative occupancy determined by relative free energy. While alternative models for translocation have not been fully developed, much of the language currently used to describe this step suggests an active mechanism coupled to conformational transitions in the **polymerase**. For example, a recent study of force generation by Escherichia coli **RNA polymerase** during transcription suggests that it is a mechanoenzyme **analogous** to kinesin of myosin motor proteins. While the proposed mechanism does not rule out conformational transitions during **polymerase** translocation, it suggests that they may be unnecessary and that translocation can be explained in terms of the affinity of the active site for nucleoside triphosphate and the relative free energies of the **polymerase** bound at different positions on the template. This mechanism makes specific predictions which are borne out experimentally with polymerases as distinct as E. coli DNAP I, phage **T7** RNAP, and E. coli RNAP.

5/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09266527 97162223 PMID: 9009210
Substrate properties of C'-methyl UTP derivatives in **T7 RNA polymerase** reactions. Evidence for N-type NTP conformation.

Tunitskaya V L; Rusakova E E; Padyukova N Sh; Ermolinsky B S; Chernyi A A ; Kochetkov S N; Lysov YuP; Mikhailov S N
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation.
FEBS letters (NETHERLANDS) Jan 6 **1997**, 400 (3) p263-6, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The number of synthetic UTP **analogues** containing methyl groups in different positions of the ribose moiety were tested as substrates for **T7 RNA polymerase** (**T7** RNAP). Two of these compounds (containing substituents in the 5' position) were shown to be weak substrates of **T7** RNAP. 3'Me-UTP was neither substrate nor inhibitor of **T7** RNAP while 2'Me-UTP was shown to terminate **RNA** chain synthesis. Conformational analysis of the **analogues** and parent nucleotide using the force-field method indicates that the allowed conformation of UTP during its incorporation into the growing **RNA** chain by **T7** RNAP is limited to the chi angle range of 192-256 degrees of N-type conformation.

5/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09256172 97173037 PMID: 9020905
Effect of aflatoxin B1-8,9-epoxide-DNA adducts on transcription of a supF gene fragment.
Yu F L; Cahill J M; Lipinski L J; Dipple A
Chemistry of Carcinogenesis Laboratory, NCI-Frederick Cancer Research and Development Center, MD 21702, USA.
Cancer letters (IRELAND) Dec 3 **1996**, 109 (1-2) p77-83, ISSN 0304-3835 Journal Code: 7600053
Contract/Grant No.: NC-CO-46000; CO; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A linearized template, obtained from the vector pGEM-3Zf(+) containing a supF gene fragment, was treated with aflatoxin B1-8,9-epoxide (AFB1 epoxide) and transcription in vitro was then studied. The template functions of both strands of the supF gene were similarly inhibited as shown by transcription with both **T7** and SP6 **RNA** polymerases. This inhibition was dose-dependent and affected the elongation step more extensively than the initiation step. Gel electrophoretic analysis of **RNA** formed by **T7 RNA polymerase** indicated that template treated with different AFB1 epoxide doses yielded the same three major truncated **RNA** fragments. Sequence analysis showed that these major sites of **RNA** truncation occurred in the vicinity of adjacent guanine residues in the template.

5/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09222909 97121268 PMID: 8961934
A direct real-time spectroscopic investigation of the mechanism of open complex formation by **T7 RNA polymerase**.
Sastry S S; Ross B M
Laboratory of Molecular Genetics, Rockefeller University, New York, New York 10021, USA. sastrys@rockvax.rockefeller.edu
Biochemistry (UNITED STATES) Dec 10 **1996**, 35 (49) p15715-25, ISSN 0006-2960 Journal Code: 0370623

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Initiation of transcription occurs through a series of steps starting with the binding of **RNA polymerase** to a promoter DNA and formation of a closed complex. The closed complexes, then isomerize to open complexes. In the open complexes a portion of the promoter DNA is unwound. Using fluorescence spectroscopy, we have investigated in real-time the mechanism of unwinding of promoter DNA during the transition from closed to open complexes of **T7 RNA polymerase**. We synthesized DNA templates containing the fluorescent base **analog** 2-aminopurine in place of adenine at specific positions in a **T7 RNA polymerase** promoter. We located the 2-aminopurine residues in the presumed melting domain of the promoter at -1, -4, and at -6. The fluorescence of 2-aminopurine increases when the DNA goes from a double-stranded form to a single-stranded form. By spectroscopically monitoring the increase in fluorescence of 2-aminopurine in DNA-**T7 RNA polymerase** complexes, we obtained kinetic and thermodynamic information for DNA unwinding. In the presence of the initiating nucleotide GTP, conformational transitions in the **polymerase**-promoter complex leading to strand opening were slower than in its absence. The rate of base pair disruption at -1, -6, and at -4 was also slower in the presence of GTP than in its absence. At 37 degrees C, base pair disruption occurred first at -1 followed by -6 and finally at -4. Open complex formation was temperature-sensitive. Temperature effects at -1, -6, and at -4 were consistent with this order of base pair disruption. The apparent activation energies (Ea) for base pair disruption around -1 and -6 were 14 kcal mol-1 and 50 kcal mol-1, respectively, also suggesting this order of base pair disruption. Transcription initiation assays using G-ladder synthesis revealed that initiation rates were almost the same on all three templates containing the modified base. Unlike strand opening, we did not observe lag times for G-ladder synthesis. We suggest that facile base pair disruption at -1 is sufficient for transcription initiation. Based on these data, it is proposed that the **polymerase** makes contacts at or near -1 and -6 resulting in untwisting of these base pairs thus creating at least two base pair disruption events at -1 and at -6, which are followed by bidirectional propagation to -4.

5/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09197184 97109091 PMID: 8951371

Incorrect base insertion and prematurely terminated transcripts during **T7 RNA polymerase** transcription elongation past benzo[a]pyrenediol epoxide-modified DNA.

Choi D J; Roth R B; Liu T; Geacintov N E; Scicchitano D A
Department of Biology, New York University, New York 10003, USA.

Journal of molecular biology (ENGLAND) Nov 29 **1996**, 264 (2) p213-9, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

DNA replication and transcription are affected adversely by the presence of bulky adducts that are generated by the covalent binding of a variety of metabolically activated environmental pollutants to cellular DNA. When these lesions are not cleared by cellular repair enzymes prior to replication, mutations and ultimately tumor initiation can occur. Transcription and DNA repair appear to be intimately connected, since certain adducts are more efficiently removed from the transcribed strands of active loci than from non-transcribed strands and other quiescent domains in the genome. The mechanism by which **RNA** polymerases deal

with bulky adducts during DNA transcription is therefore of great interest. The availability of site-specifically modified and stereochemically defined oligodeoxyribonucleotides derived from the covalent reaction of 7r, 8t-dihydroxy-9, 10t-epoxy- 7,8,9,10-tetrahydrobenzo[a]pyrene (anti-BPDE) with guanine residues prompted us to study the efficiencies of transcription past these lesions using bacteriophage **T7 RNA polymerase**. We show here that **T7 RNA polymerase** can bypass such lesions in a DNA template, providing that a cytosine residue is incorporated opposite anti-BPDE-modified guanine. However, when an incorrect base (most frequently a purine) is inserted opposite the modified site, the **RNA polymerase** stalls, and the complex dissociates, resulting in a truncated transcript. The ability of the **T7 RNA polymerase** to discriminate between a correct and an incorrect inserted base and, accordingly, to continue or terminate transcription, might constitute an important mechanism that ensures the fidelity of transcription past a modified base present on the transcribed strand of the DNA template.

5/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09172830 97085415 PMID: 8931555
Thermodynamic and kinetic measurements of promoter binding by **T7 RNA polymerase.**
Ujvari A; Martin C T
Department of Chemistry, University of Massachusetts, Amherst 01003-4510, USA.
Biochemistry (UNITED STATES) Nov 19 **1996**, 35 (46) p14574-82, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previous steady state kinetic studies of the initiation of transcription by **T7 RNA polymerase** have shown that melting of the DNA helix near the transcription start site is not rate limiting [Maslak, M., & Martin, C. T. (1993) Biochemistry 32, 4281-4285]. In the current work, fluorescence changes in a nucleotide **analog** incorporated within the promoter are used to monitor changes in the DNA helix associated with **polymerase** binding. The fluorescence of 2-aminopurine has been previously shown to depend on the environment of the base, with fluorescence increasing in the transition from a double-stranded to a single-stranded environment [Xu, D., Evans, K.O., & Nordlund, T. M.(1994) Biochemistry 33, 9592-9599]. Fluorescence changes associated with **polymerase** binding to promoters incorporating 2-aminopurine at positions -4 through -1 support a model which includes melting, in the statically bound complex, of the region of the promoter near the start site. Equilibrium titrations at 25 degrees C with label at position -2 provide a thermodynamic measure of the dissociation constant (Kd = 4.8 nM) for promoter binding, while stopped-flow kinetic assays measure the apparent association (k1 = 5.6 x 10(7) M-1 s-1) and dissociation (k-1 = 0.20 s-1) rate constants for simple promoter binding (the ratio k-1/k1 = 3.6 nM, in good agreement with the thermodynamic measurement of Kd). These results suggest that binding is close to the diffusion-controlled limit and helix melting is extremely rapid. In studies of structurally altered promoters, a base functional group substitution at position -10 is shown to significantly decrease k1, with little effect on k-1. In contrast, removal of the nontemplate strand from position +1 downstream results in a large decrease in k-1, with no significant effect on k1.

5/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09165318 97080525 PMID: 8921870
Cloning, sequencing, overexpression in Escherichia coli, and inactivation of the valine dehydrogenase gene in the polyether antibiotic producer Streptomyces cinnamonensis.
Leiser A; Birch A; Robinson J A
Institute of Organic Chemistry, University of Zurich, Switzerland.
Gene (NETHERLANDS) Oct 24 **1996**, 177 (1-2) p217-22, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The catabolism of branched chain amino acids, especially valine, appears to play an important role in furnishing building blocks for macrolide antibiotic biosynthesis. To determine for the first time the importance of valine dehydrogenase (vdh) in polyether antibiotic biosynthesis, the vdh gene from Streptomyces cinnamonensis has been cloned and sequenced. The enzyme (M(r)37,718 Da) has been produced in large amounts in an active form in the E. coli cytoplasm using a **T7 RNA-polymerase** expression system. Upon inactivation of the gene in S. cinnamonensis by a double-crossover mechanism, a hyg::vdh mutant was isolated that was devoid of vdh activity. Upon growth in chemically defined media, as well as a complex medium optimised for monensin production, the mutant and wild-type grew equally well and reached the same levels of monensin production. In both strains a valine transaminase activity could be detected that provides an alternative route for converting valine into 2-oxoisovaleric acid. The results show that vdh is not essential for normal growth of S. cinnamonensis, and its inactivation does not significantly affect normal levels of monensin production in this strain.

5/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09134254 97040518 PMID: 8885831
Identification of the template-binding cleft of **T7 RNA polymerase** as the site for promoter binding by photochemical cross-linking with psoralen.
Sastry S S
Laboratory of Molecular Genetics, Rockefeller University, New York, New York 10021, USA. sastrys@rockvax.rockefeller.edu
Biochemistry (UNITED STATES) Oct 22 **1996**, 35 (42) p13519-30, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We describe a novel method of photo-cross-linking DNA-binding proteins to DNA employing psoralen as a tether. We apply this method for the interaction of **T7 RNA polymerase** to its promoter. The crystallographic model of **T7 RNA polymerase** shows a cleft formed by the palm, thumb, and fingers domains. It was proposed that template DNA binds in the cleft. Here we directly and positively identify, in solution, the cleft as the seat of template binding. We photo-cross-linked a 23 bp promoter DNA to **T7 RNA polymerase** . We then determined the masses of cross-linked tryptic peptides by mass spectrometry and analyzed their amino acid composition. The cross-linked peptides were projected on the crystal structure of **T7 RNA polymerase** . The peptides nicely decorated the back, front, and side wall of the cleft. In a previous work [Sastry et al. (1993) Biochemistry 32, 5526-5538] we used site-specific psoralen furan-side monoadducts for cross-linking DNAs to DNA-binding proteins. We cross-linked a single-stranded 12-mer oligonucleotide to **T7 RNA**

polymerase . We isolated and purified a DNA cross-linked tryptic peptide. We then used mass spectrometry and amino acid composition analysis to identify the location of this peptide on the **T7 RNA polymerase** primary sequence. In the present work we have mapped this peptide on the 3-D structure of **T7 RNA polymerase**. This peptide maps in the fingers domain of the **polymerase**. On the basis of a comparison of the map positions of peptides that cross-linked to either promoter DNA or single-stranded oligo-DNA, we propose that different functional domains may be involved in binding of double-stranded promoter DNA and nonspecific single-stranded DNA. Whereas the cleft of the **polymerase** is the seat of double-stranded promoter binding, the fingers domain may be used by the **polymerase** to grab single-stranded DNA (or **RNA**) in a nonspecific manner. Alternatively, the single-stranded oligo binding site may be an **RNA** product-binding site during transcription. The photochemical techniques we have developed [Sastry et al. (1993) Biochemistry 32, 5526-5538; this work] can be applied to other DNA-protein complexes to map DNA-binding domains.

5/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09068615 96419174 PMID: 8821947
Purification of the Tn10-specified tetracycline efflux antiporter TetA in a native state as a polyhistidine fusion protein.
Aldema M L; McMurry L M; Walmsley A R; Levy S B
Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.
Molecular microbiology (ENGLAND) Jan **1996**, 19 (1) p187-95,
ISSN 0950-382X Journal Code: 8712028
Contract/Grant No.: AI30646; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The bacterial tetracycline-resistance determinant from Tn10 encodes a 43 kDa membrane protein, TetA, responsible for active efflux of tetracyclines. The tetA gene was cloned behind a **T7** promoter/lac operator in a plasmid that provided fusion of TetA to a polyhistidine-carboxy terminal tail. A second plasmid provided a regulated **T7 RNA polymerase** . The specific activity of the TetA fusion protein was between 10-40% that of the wild-type protein as assayed by tetracycline resistance in cells and by transport in membrane vesicles. The fusion protein, overproduced approximately 3-13-fold, was purified by nickel chelation chromatography. Calculations from circular dichroism spectra of the purified protein solubilized in dodecylmaltoside gave an alpha-helix content of 54-64%, close to the 68% predicted from the amino acid sequence by hydropathy analysis (12 membrane-spanning helices) for the native protein in the membrane bilayer. Fluorescence studies showed binding activity of the purified protein to its substrate, the tetracycline **analogue** 13-(cyclopentylthio)-5-hydroxy-6-alpha-deoxytetracycline. These findings suggested that the purified protein was in a native state.

5/3,AB/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09055291 96410760 PMID: 8812830
Overproduction and purification of sigmaS, the Escherichia coli stationary phase specific sigma transcription factor.
Nguyen L H; Burgess R R
McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, Wisconsin, 53706, USA.
Protein expression and purification (UNITED STATES) Aug **1996**, 8

(1) p17-22, ISSN 1046-5928 Journal Code: 9101496
Contract/Grant No.: CA07175; CA; NCI; GM28575; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This paper reports the overproduction and the details of a rapid method to purify active sigmaS monomers from a **T7 RNA polymerase** -based protein expression system. This 2-day procedure involves solubilizing inclusion bodies in sarkosyl detergent, removal of sarkosyl by dialysis, and a single gel filtration column chromatography step. The final yield of sigmaS is about 9 mg of approximately 92% purity from 0.5 g of wet weight cells. Overproduced sigmaS binds to core **RNA polymerase** and supports transcription from the bolAp1 promoter, a sigmaS-dependent promoter.

5/3,AB/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09026161 96394453 PMID: 8798559
Purification and characterization of the vaccinia virus deoxyuridine triphosphatase expressed in Escherichia coli.
Roseman N A; Evans R K; Mayer E L; Rossi M A; Slabaugh M B
Department of Biology, Williams College, Williamstown, Massachusetts 01267, USA.
Journal of biological chemistry (UNITED STATES) Sep 20 **1996**, 271 (38) p23506-11, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: A124294; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The deoxyuridine triphosphatase gene of vaccinia virus, encoded by the open reading frame F2L, was cloned into Escherichia coli and expressed under the control of a bacteriophage **T7** promoter. After induction of **T7 RNA polymerase** by isopropyl beta-D-thiogalactopyranosi de, a 16.5-kDa peptide accumulated to high levels. This 16.5-kDa protein was purified to homogeneity and characterized. Gel filtration of the purified protein revealed a trimeric native structure. Biochemical analysis revealed the enzyme to be a metalloenzyme; enzymatic activity is inhibited by EDTA. This inhibition was reversed by the addition of Mg2+, Mn2+, or Zn2+. While the enzyme activity was highly specific for dUTP with an apparent Km of 0.94 microM, inhibition studies show that 8-azido-ATP acted as a competitive inhibitor of dUTP with a Ki of approximately 173 microM. Also, protection studies demonstrated that nucleotide competitors inhibit photoincorporation of the photoaffinity **analogues** [gamma-32P]5-azido- dUTP and [gamma-32P]8-azido-ATP. This suggests that while catalytic activity is limited to dUTP, other nucleotides can bind the active site.

5/3,AB/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09020458 96374490 PMID: 8780781
Efficient inhibition of transcription elongation in vitro by oligonucleotide phosphoramidates targeted to proviral HIV DNA.
Giovannangeli C; Perrouault L; Escude C; Gryaznov S; Helene C
Laboratoire de Biophysique Museum National d'Histoire Naturelle, INSERM U.201-CNRS URA 481, Paris, France.
Journal of molecular biology (ENGLAND) Aug 23 **1996**, 261 (3) p386-98, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed

Triplex-forming oligophosphoramidates containing thymines and cytosines or 5-methyl cytosines (5' T4CT4C6T 3') bind strongly to a 16 basepair oligopurine.oligopyrimidine sequence of HIV proviral DNA even at neutral pH. These triple-helical complexes formed with oligonucleotide **analogues** with N3'-->P5' phosphoramidate linkages are remarkably stable compared to oligonucleotides with natural phosphodiester linkages. In transcription assays the (T,C)-containing phosphoramidate oligomers induce an efficient arrest of both bacteriophage and eukaryotic transcriptional machineries under conditions where the isosequential phosphodiesters have no inhibitory effect. In both cases the **RNA polymerase** (SP6, **T7** or Pol II) is physically blocked by the non-covalent triplex and **RNA** synthesis is stopped at the triplex site. However the eukaryotic transcription machinery is blocked more efficiently (at submicromolar concentration) than the bacteriophage polymerases. The analysis of the 3'-ends of the truncated transcripts provides evidence for differences in the termination patterns induced by the triplex barrier for the bacteriophage and the eukaryotic systems. This in vitro comparative study provides the basis for the rational design of strong transcriptional inhibitors. The efficient in vitro inhibition obtained using the phosphoramidate oligomers in the eukaryotic transcription assay makes them good candidates for the development of sequence-specific antigene agents.

5/3,AB/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08933605 96283646 PMID: 8670848

Template-free generation of **RNA** species that replicate with bacteriophage **T7 RNA polymerase**.

Biebricher C K; Luce R
Max-Planck-Institute for Biophysical Chemistry, Gottingen, Germany.

EMBO journal (ENGLAND) Jul 1 **1996**, 15 (13) p3458-65, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A large variety of different **RNA** species that are replicated by DNA-dependent **RNA polymerase** from bacteriophage **T7** have been generated by incubating high concentrations of this enzyme with substrate for extended time periods. The products differed from sample to sample in molecular weight and sequence, their chain lengths ranging from 60 to 120. The mechanism of autocatalytic amplification of **RNA** by **T7 RNA polymerase** proved to be **analogous** to that observed with viral **RNA**-dependent **RNA** polymerases (replicases): only single-stranded templates are accepted and complementary replica strands are synthesized. With enzyme in excess, exponential growth was observed; linear growth resulted when the enzyme was saturated by **RNA** template. The plus strands, present at 90% of the replicating **RNA** species, were found to have GG residues at both termini. Consensus sequences were not found among the sequences of the replicating **RNA** species. The secondary structures of all species sequenced turned out to be hairpins. The **RNA** species were specifically replicated by **T7 RNA polymerase**; they were not accepted as templates by the **RNA** polymerases from Escherichia coli or bacteriophage SP6 or by Qbeta replicase; T3 **RNA polymerase** was partially active. Template-free production of **RNA** was completely suppressed by addition of DNA to the incubation mixture. When both DNA and **RNA** templates were present, transcription and replication competed, but **T7 RNA polymerase** preferred DNA as a template. No replicating **RNA** species were detected in vivo in cells expressing **T7**

RNA polymerase.

5/3,AB/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08902725 96251084 PMID: 8668534
Non-hydrogen bonding 'terminator' nucleosides increase the 3'-end homogeneity of enzymatic **RNA** and DNA synthesis.
Moran S; Ren R X; Sheils C J; Rumney S; Kool E T
Department of Chemistry, University of Rochester, Rochester, NY 14627, USA.
Nucleic acids research (ENGLAND) Jun 1 **1996**, 24 (11) p2044-52, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM 52956; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We report the use of novel non-polar nucleoside **analogues** as terminators of enzymatic **RNA** and DNA synthesis. Standard 'runoff' **RNA** synthesis by **T7 RNA polymerase** gives **RNA** products which have ragged ends as a result of transcription which often extends beyond the end of the template DNA strand. Similarly, the Klenow fragment of Escherichia coli DNA **polymerase** I tends to run past the end of the template strand during DNA synthesis. We report here that certain non-hydrogen-bonding nucleoside **analogues**, when placed at the downstream 5'-end of a template DNA strand, cause the polymerases to stop more abruptly at the last coding nucleotide. This results in a considerably more homogeneous oligonucleotide being produced. Three novel nucleosides are tested as potential terminators: 4-methylindole beta-deoxynucleoside (M), 1-naphthyl alpha-deoxynucleoside (N) and 1-pyrenyl alpha-deoxynucleoside (P). Comparison is made to an abasic nucleoside (phi) and to unterminated synthesis. Of these, M is found to be the most efficient at terminating transcription, and both P and M are highly effective at terminating DNA synthesis. It is also found that the ability of a nucleoside to stall synthesis when it is internally placed in the template strand is not necessarily a good predictor of terminating ability at the end of a template. Such terminator nucleosides may be useful in the preparative enzymatic synthesis of **RNA** and DNA, rendering purification simpler and lowering the cost of synthesis by preventing the uptake of potentially costly nucleotides into unwanted products.

5/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08872790 96241608 PMID: 8639503
Sequence-specific actinomycin D binding to single-stranded DNA inhibits HIV reverse transcriptase and other polymerases.
Rill R L; Hecker K H
Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, 32306-3006, USA.
Biochemistry (UNITED STATES) Mar 19 **1996**, 35 (11) p3525-33, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Primer extension assays using recombinant templates constructed to contain all 256 possible base quartets in a minimum length sequence were used to examine binding of the anticancer drug actinomycin D to single-stranded DNA. Single-stranded templates were generated by digestion of linearized plasmid with the double-strand-specific **T7** gene 6

exonuclease. Actinomycin D formed high-affinity, kinetically stable complexes that paused primer elongation at specific sites by HIV-1 reverse transcriptase, Sequenase (modified T4 DNA **polymerase**), the Klenow fragment of Escherichia coli DNA **polymerase**, and Vent (exo-) DNA **polymerase** . Pauses occurred most commonly near G+C-rich nucleotide clusters, including GpC steps, the preferred sites of double-stranded DNA binding. Complexes were stable for several minutes at temperatures over 50 degrees C as determined by their abilities to pause Vent **polymerase** at elevated temperatures. Significant variations were noted in pause patterns of different polymerases, demonstrating differential responses of polymerases to a bound actinomycin. Covalent adducts formed on template DNA by a photoaffinity **analog** of actinomycin D completely stopped primer extension. These results support the possibility that actinomycin D inhibits transcription elongation by complexing single-stranded DNA in the open transcription complex. Single-stranded DNA binding by actinomycin D or **analogs** may also provide routes for combating HIV or other viruses which replicate through single-stranded intermediates.

5/3,AB/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08827810 96183488 PMID: 8619992
Major groove recognition elements in the middle of the **T7 RNA polymerase** promoter.
Li T; Ho H H; Maslak M; Schick C; Martin C T
Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003-4510, USA.
Biochemistry (UNITED STATES) Mar 26 **1996**, 35 (12) p3722-7, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
T7 RNA polymerase recognizes a relatively small promoter extending only 17 base pairs upstream from the start site for transcription. A model for this recognition suggests that the enzyme interacts with the major groove of duplex DNA in the region centered at position -9 [Muller, D.K., et al. (1989) Biochemistry 28, 3306-3313], and recent kinetic analyses of promoters containing base **analogs** at positions -10 and -11 have provided support for this model [Schick, C., & Martin, C.T. (1993) Biochemistry 32, 4275-4280; Schick, C., & Martin, C.T. (1995) Biochemistry 34, 666-672]. In the current work, we extend this analysis across the proposed major groove, identifying specific base functional group contacts at positions -9 through -5. Specifically, the 6-carbonyl of guanine at positions -9 and -7, the 6-amino group of adenine at position -8, the 5-methyl group of thymine at position -6 and the 2-amino group of guanine at position -5 are identified as primary contacts. The results strongly support the model for duplex recognition in this region of the promoter and suggest that recognition continues along one face of the helix beyond the major groove and into the adjoining minor groove at position -5, where helix melting begins.

5/3,AB/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08733731 96069381 PMID: 8524238
RNA polymerase bypass at sites of dihydrouracil: implications for transcriptional mutagenesis.
Liu J; Zhou W; Doetsch P W
Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322, USA.
Molecular and cellular biology (UNITED STATES) Dec **1995**, 15

(12) p6729-35, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: CA55896; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Dihydrouracil (DHU) is a major base damage product formed from cytosine following exposure of DNA to ionizing radiation under anoxic conditions. To gain insight into the DNA lesion structural requirements for **RNA polymerase** arrest or bypass at various DNA damages located on the transcribed strand during elongation, DHU was placed onto promoter-containing DNA templates 20 nucleotides downstream from the transcription start site. In vitro, single-round transcription experiments carried out with SP6 and **T7 RNA** polymerases revealed that following a brief pause at the DHU site, both enzymes efficiently bypass this lesion with subsequent rapid generation of full-length runoff transcripts. Direct sequence analysis of these transcripts indicated that both **RNA** polymerases insert primarily adenine opposite to the DHU site, resulting in a G-to-A transition mutation in the lesion bypass product. Such bypass and insertion events at DHU sites (or other types of DNA damages), if they occur in vivo, have a number of important implications for both the repair of such lesions and the DNA damage-induced production of mutant proteins at the level of transcription (transcriptional mutagenesis).

5/3,AB/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08724000 96074606 PMID: 7479898
Catalytic editing properties of DNA polymerases.
Canard B; Cardona B; Sarfati R S
Faculte de Medecine, Unite de Recherche Associee-Centre National de la Recherche Scientifique 1462, Nice, France.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 21 **1995**, 92 (24) p10859-63, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Enzymatic incorporation of 2',3'-dideoxynucleotides into DNA results in chain termination. We report that 3'-esterified 2'-deoxynucleoside 5'-triphosphates (dNTPs) are false chain-terminator substrates since DNA polymerases, including human immunodeficiency virus reverse transcriptase, can incorporate them into DNA and, subsequently, use this new 3' end to insert the next correctly paired dNTP. Likewise, a DNA substrate with a primer chemically esterified at the 3' position can be extended efficiently upon incubation with dNTPs and **T7** DNA **polymerase** lacking 3'-to-5' exonuclease activity. This enzyme is also able to use dTTP-bearing reporter groups in the 3' position conjugated through amide or thiourea bonds and cleave them to restore a DNA chain terminated by an amino group at the 3' end. Hence, a number of DNA polymerases exhibit wide catalytic versatility at the 3' end of the nascent DNA strand. As part of the polymerization mechanism, these capabilities extend the number of enzymatic activities associated with these enzymes and also the study of interactions between DNA polymerases and nucleotide **analogues**.

5/3,AB/49 (Item 49 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08660942 96017728 PMID: 7563075
Regions of 23 S ribosomal **RNA** proximal to transfer **RNA** bound

at the P and E sites.
Bullard J M; van Waes M A; Bucklin D J; Hill W E
Division of Biological Sciences, University of Montana, Missoula 59812, USA.
Journal of molecular biology (ENGLAND) Oct 6 **1995**, 252 (5) p572-82, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM35717; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
tRNAPhe transcribed in a **T7 RNA polymerase** system has been modified in such a way that 4-thiouridines have randomly replaced unmodified uridines. These 4-thiouridines serve as sites for conjugation of the cleavage reagent 5-iodoacetamido-1,10-phenanthroline (IOP). 1,10-Phenantholine, when complexed with Cu2+ in a reducing environment, causes hydrolysis of nearby nucleic acids. We show here that tRNA-phenanthroline (tRNA-OP) conjugates, when bound in situ to the P- and E-sites of 70 S ribosomes, cause cleavage, mainly in domains I, III and V of 23 S ribosomal **RNA** (rRNA). The cleavage sites in domain V predominantly occur very close to or in the peptidyl-transferase region. The regions of domain I and III that are cleaved are apparently folded in the 50 S ribosomal subunit so as to be proximal to the peptidyl-transferase center. Most of the cleavage events occur whether the tRNA-OP conjugate is bound to ribosomes alone, or yeast tRNA is also present in the P/P hybrid state. Cleavages that occur only in the absence of yeast tRNA are limited to the 1100 region of domain II, and the 2800 region of domain VI. Cleavages that occur only in the presence of yeast occur in the 2170 region of domain V. The regions of 23 S rRNA in which tRNA-OP induced cleavage occur complement those sites shown by chemical protection and cross-liking to be in a close proximity to the tRNA. However, the cleavage approach allows a more versatile and expanded view of the near neighborhood of rRNA surrounding the tRNA. These results provide considerable information which will allow a more detailed modeling of the tertiary structure of the 50 S ribosomal subunit.

5/3,AB/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08619908 95377293 PMID: 7649163
Overproduction in Escherichia coli, purification and properties of human prothymosin alpha.
Evstafieva A G; Chichkova N V; Makarova T N; Vartapetian A B; Vasilenko A V; Abramov V M; Bogdanov A A
A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia.
European journal of biochemistry / FEBS (GERMANY) Aug 1 **1995**, 231 (3) p639-43, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A bacterial strain overproducing human prothymosin alpha was constructed based on the efficient **T7 RNA polymerase** transcription of human prothymosin alpha cDNA. The highest yield of the human prothymosin alpha, up to 30% of the total bacterial protein, was achieved with constructions containing 6-10 nucleotides between the Shine-Dalgarno sequence and initiation ATG codon. Unexpectedly, cells grown in the presence of inducer of **T7 RNA polymerase** synthesis produced substantially lower levels of prothymosin alpha than those grown in the absence of inducer. A simple procedure for prothymosin alpha isolation was elaborated, resulting in large amounts of electrophoretically pure and immunoactive protein.

5/3,AB/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08604694 95363981 PMID: 7637014
RNA replication by respiratory syncytial virus (RSV) is directed by the N, P, and L proteins; transcription also occurs under these conditions but requires RSV superinfection for efficient synthesis of full-length mRNA.
Grosfeld H; Hill M G; Collins P L
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disease, Bethesda, Maryland 20892-0720, USA.
Journal of virology (UNITED STATES) Sep **1995**, 69 (9) p5677-86, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previously, a cDNA was constructed so that transcription by **T7 RNA polymerase** yielded a approximately 1-kb negative-sense **analog** of genomic **RNA** of human respiratory syncytial virus (RSV) containing the gene for chloramphenicol acetyltransferase (CAT) under the control of putative RSV transcription motifs and flanked by the RSV genomic termini. When transfected into RSV-infected cells, this minigenome was "rescued," as evidenced by high levels of CAT expression and the production of transmissible particles which propagated and expressed high levels of CAT expression during serial passage (P.L. Collins, M. A. Mink, and D. S. Stec, Proc. Natl. Acad. Sci. USA, 88:9663-9667, 1991). Here, this cDNA, together with a second one designed to yield an exact-copy positive-sense RSV-CAT **RNA** antigenome, were each modified to contain a self-cleaving hammerhead ribozyme for the generation of a nearly exact 3' end. Each cDNA was transfected into cells infected with a vaccinia virus recombinant expressing **T7 RNA polymerase**, together with plasmids encoding the RSV N, P, and L proteins, each under the control of a **T7** promoter. When the plasmid-supplied template was the mini-antigenome, the minigenome was produced. When the plasmid-supplied template was the minigenome, the products were mini-antigenome, subgenomic polyadenylated mRNA and progeny minigenome. Identification of progeny minigenome made from the plasmid-supplied minigenome template indicates that the full RSV **RNA** replication cycle occurred. **RNA** synthesis required all three RSV proteins, N, P, and L, and was ablated completely by the substitution of Asn for Asp at position 989 in the L protein. Thus, the N, P, and L proteins were sufficient for the synthesis of correct minigenome and antigenome, but this was not the case for subgenomic mRNA, indicating that the requirements for **RNA** replication and transcription are not identical. Complementation with N, P, and L alone yielded an mRNA pattern containing a large fraction of molecules of incomplete, heterogeneous size. In contrast, complementation with RSV (supplying all of the RSV gene products) yielded a single discrete mRNA band. Superinfection with RSV of cells staging N/P/L-based **RNA** synthesis yielded the single discrete mRNA species. Some additional factor supplied by RSV superinfection appeared to be involved in transcription, the most obvious possibility being one or more additional RSV gene products.

5/3,AB/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08586457 95343550 PMID: 7618275
RNA transcripts derived from a cloned full-length copy of the feline calicivirus genome do not require VpG for infectivity.
Sosnovtsev S; Green K Y

Laboratory of Infectious Diseases, National Institute of Allergies and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.
Virology (UNITED STATES) Jul 10 **1995**, 210 (2) p383-90, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Feline calicivirus (FCV) is a positive-strand, nonenveloped **RNA** virus in the family Caliciviridae. A cDNA library of the Urbana (URB) strain of FCV was generated and the sequence of the genome was determined from overlapping clones except for 13 bases from the 5'-end. The 5'-end sequence was identified by analysis of clones derived by RT-PCR across the ligated 5'- and 3'-ends of the **RNA** genome. A full-length cDNA clone of the **RNA** genome of the URB strain was constructed and placed downstream of the **T7 RNA polymerase** promoter and **RNA** transcripts generated in vitro from this clone were infectious when introduced into feline kidney cells. A virus-encoded genome-linked protein, VpG, which is considered to be essential for infectivity of wild-type genomic FCV **RNA**, was not required for the initiation of FCV infection by the synthetic transcripts. However, the addition of a cap structure **analog** (m7G(5')ppp(5')G) during in vitro transcription of the synthetic **RNA** was necessary for successful virus recovery. Two silent mutations engineered into the full-length clone were identified in the genomic **RNA** from recovered progeny virus. This system of introducing site-specific genetic changes into the genome of feline calicivirus and the recovery of infectious mutant viruses will enable studies related to the molecular basis for replication, growth restriction, and pathogenicity of this and other members of the Caliciviridae.

5/3,AB/53 (Item 53 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08503079 95258334 PMID: 7537876
Preparation of probe-modified **RNA** with 5-mercapto-UTP for analysis of protein-**RNA** interactions.
He B; Riggs D L; Hanna M M
Department of Botany/Microbiology, University of Oklahoma, Norman 73019, USA.
Nucleic acids research (ENGLAND) Apr 11 **1995**, 23 (7) p1231-8, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: RO1GM47493; GM; NIGMS; RO1GM47881; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We report a modified synthesis for 5-mercapto-UTP (5-SH-UTP) and its use for analysis of protein-**RNA** interactions utilizing Escherichia coli and **T7 RNA** polymerases and yeast **RNA** polymerases I and III. 5-SH-UTP did not affect transcriptional pausing, Rho-independent termination or recognition of the E. coli transcription complex by NusA. **RNA** containing 5-SH-UMP did not crosslink to **polymerase** when irradiation was 302 or 337 nm. Transcription complexes containing **RNA** substituted with 5-SH-UMP were post-transcriptionally modified to attach a photocross-linking group to thiol-tagged nucleotides in the **RNA** on the surface of the **polymerase** of free in solution. The pKa for 5-SH-UTP was determined to be 5.6, so modification of the thiol groups in the **RNA** with p-azidophenacyl bromide could be carried out at pH 7. Addition of the transcription termination factor Rho, a **RNA** binding protein, to E. coli transcription complexes resulted in **RNA** crosslinking to Rho and to the beta and beta' subunits of **polymerase** . Using 5-SH-UTP, one can distinguish **RNA** binding domains on the

surface of **RNA** polymerases or other **RNA** binding proteins from those buried within the protein.

5/3,AB/54 (Item 54 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08496562 95249382 PMID: 7731814
In vitro transcription close to the melting point of DNA: analysis of Thermotoga maritima **RNA polymerase** -promoter complexes at 75 degrees C using chemical probes.
Meier T; Schickor P; Wedel A; Cellai L; Heumann H
Max Planck Institute for Biochemistry, Martinsried, Germany.
Nucleic acids research (ENGLAND) Mar 25 **1995**, 23 (6) p988-94, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The interaction of DNA dependent **RNA polymerase** of the extreme thermophile bacteria Thermotoga maritima with a promoter bearing DNA fragment was investigated in the temperature range from 20 to 85 degrees C. We show that the T. maritima **RNA polymerase** recognizes and utilizes the Escherichia coli **T7** A1 promoter with an efficiency similar to that of the E. coli **polymerase**. We have investigated the interaction of both polymerases with the same promoter over a wide range of temperatures using hydroxyl radical foot-printing and osmium tetroxide probing. This study revealed that the T. maritima **polymerase** goes through a series of isomerisation events very similar to the E. coli **polymerase**, i.e. the closed, intermediate and open complexes, but the transitions themselves occur at radically different temperatures. This indicates that conformational changes in the DNA that accompany initiation of transcription such as promoter melting are determined by the **polymerase** rather than the DNA sequence.

5/3,AB/55 (Item 55 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08482191 95239732 PMID: 7536848
NusA contacts nascent **RNA** in Escherichia coli transcription complexes.
Liu K; Hanna M M
Department of Botany and Microbiology, University of Oklahoma, Norman 73019 USA.
Journal of molecular biology (ENGLAND) Apr 7 **1995**, 247 (4) p547-58, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: RO1 GM47493; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have examined the interaction between NusA and the nascent **RNA** in Escherichia coli transcription complexes on four different templates. Photocrosslinking CTP and UTP **analogs** were incorporated internally and at the 3' end of the **RNA**. Identical templates with and without boxA sequences were compared. We found that NusA did not contact the ten nucleotides nearest to the 3' end of the **RNA** in complexes containing **RNA** up to 20 nucleotides long. Longer **RNA** did crosslink to NusA with all four templates examined, however. We reported that **RNA** 80 nucleotides long from the bacteriophage **T7** A1 promoter substituted in two **RNA** stem-loops with photocrosslinking UMP **analogs** did not crosslink to NusA, even though interaction between NusA and the transcription complex were demonstrated. Here, we report that when this

same **RNA** is substituted at CMP residues, it does crosslink to NusA. Templates containing the E. coli ribosomal **RNA** promoter rrnG P2, with and without a boxA sequence downstream, were compared. Long RNAs from both crosslinked to NusA, and thus boxA **RNA** sequences are not required for interaction with NusA. NusA did not interact with the free **RNA** containing boxA once released from the transcription complex, nor did it interact with **RNA** in a binary complex containing only **RNA** **polymerase** and **RNA**, without the DNA template.

5/3,AB/56 (Item 56 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08471596 95223961 PMID: 7708666
Total chemical synthesis of a ribozyme derived from a group I intron.
Whoriskey S K; Usman N; Szostak J W
Department of Molecular Biology, Massachusetts General Hospital, Boston 02114, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 28 **1995**, 92 (7) p2465-9, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: GM45315; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We describe the complete chemical synthesis of a ribozyme that catalyzes template-directed oligonucleotide ligation. The specific activity of the synthetic ribozyme is nearly identical to that of the same enzyme generated by in vitro transcription with **T7 RNA polymerase**. The ribozyme is derived from a group I intron and consists of three **RNA** fragments of 36, 43, and 59 nt that self-assemble to form a catalytically active complex. We have site-specifically substituted ribonucleotide **analogs** into this enzyme and have identified two 2'-hydroxyl groups that are required for full catalytic activity. In contrast, neither the 2'-hydroxyl nor the exocyclic amino group of the conserved guanosine in the guanosine binding site is necessary for catalysis. By allowing the ribozyme to be modified as easily as its substrates, this synthetic ribozyme system should be useful for testing specific hypotheses concerning ribozyme-substrate interactions and tertiary interactions within the ribozyme.

5/3,AB/57 (Item 57 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08367425 95119054 PMID: 7819262
Tests of a model of specific contacts in **T7** **RNA** **polymerase**-promoter interactions.
Schick C; Martin C T
Program in Molecular and Cellular Biology, University of Massachusetts, Amherst 01003.
Biochemistry (UNITED STATES) Jan 17 **1995**, 34 (2) p666-72, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The **T7**, T3, and SP6 **RNA** polymerases represent a highly homologous family of enzymes that recognize similarly homologous promoter DNA sequences. Despite these similarities, the enzymes are highly specific for their respective promoters. Studies of mutant **RNA** polymerases have linked a specific amino acid residue in the protein to recognition of bases at positions -11 and -10 in the promoter [Raskin, C. A., et al.

(1992) J. Mol. Biol. 228, 506-515]. In kinetic analyses of transcription from synthetic promoters containing base-**analog** substitutions, we have recently shown that at positions -11 and -10 of the T3 promoter, T3 **RNA polymerase** recognizes functional groups along the nontemplate strand wall of the major groove [Schick, C., & Martin, C. T. (1993) Biochemistry 32, 4275-4780]. We now extend these studies to the homologous region of the **T7** promoter. The results confirm extrapolations from the T3 system and show that **T7 RNA polymerase** recognizes corresponding functional groups at positions -11 and -10 of the **T7** promoter. The results are consistent with a direct readout model for recognition of these bases [Raskin, C. A., et al. (1992) J. Mol. Biol., 228, 506-515], in which the 6-carbonyl and 7-imino groups of the nontemplate guanine at position -11 and the 6-amino group of the nontemplate adenine at position -10 of the **T7** promoter are directly involved in binding. The results further support an overall model for promoter recognition in which the enzyme binds to one face of the duplex DNA in this upstream region of the promoter.

5/3,AB/58 (Item 58 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08352909 95112847 PMID: 7813472

Inhibition of **T7 RNA polymerase** transcription by phosphate and phosphorothioate triplex-forming oligonucleotides targeted to a R.Y site downstream from the promoter.

Alunni-Fabbroni M; Manfioletti G; Manzini G; Xodo L E

Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Italy.

European journal of biochemistry / FEBS (GERMANY) Dec 15 **1994**, 226 (3) p831-9, ISSN 0014-2956 Journal Code: 0107600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The effect of triplex-forming oligonucleotides (TFO) on the transcription activity of **T7 RNA polymerase** has been investigated by an in vitro assay. The TFOs, either containing only phosphate (PO2) or phosphate and phosphorothioate (POS) internucleotide linkages, were targeted to a 30-bp homopurine. homopyrimidine (R.Y) site cloned in plasmid Bluescript KS+ about four helical turns downstream from the **T7 RNA** promoter. Band-shift and ultraviolet absorption melting experiments showed that the designed pyrimidine PO2 and POS TFOs form stable triple-helical complexes with the R.Y target duplex (the delta GTFO values of triplex formation vary from -42 to -63 kJ/mol). The triple-helical complexes resulting from POS oligonucleotides were less stable (by 4-12 kJ/mol) than those obtained with PO2 **analogues**, the magnitude of destabilization being dependent on the number of POS groups present in the third strand. The designed TFOs were shown to efficiently repress bacteriophage **T7 RNA polymerase** transcription under different experimental conditions. The repression depended on pH, TFO concentration and temperature. When the TFO/template ratio was fixed to 100, a strong repressive effect was observed with normal and phosphorothioate pyrimidine TFOs, also under physiological conditions. In contrast, a purine-rich oligonucleotide containing 44% of guanine residues promoted only a weak transcription inhibition, even at a TFO/template ratio as high as 750. Both PO2- and POS-containing pyrimidine TFOs produced their strong repressive effect on **T7 RNA polymerase** transcription even when they were added to the reaction mixture simultaneously with the **polymerase** . A mechanism of transcription repression is discussed. The data reported in this paper are useful for designing oligonucleotides acting as artificial repressors in the antigene strategy and indicate that the R.Y target need not to be precisely confined to the promoter.

superficially, resemble translational decoding. We report here that an oligoribonucleotide **analogue** of the decoding region interacts with both antibiotic and **RNA** ligands of the 30S subunit in a manner that correlates with normal subunit function. The activities of the decoding region **analogue** suggest that the intimidating structural complexity of the ribosome can be, to some degree, circumvented.

5/3,AB/61 (Item 61 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08180768 94316683 PMID: 8041784
Controlled expression of plastid transgenes in plants based on a nuclear DNA-encoded and plastid-targeted **T7 RNA polymerase**.
McBride K E; Schaaf D J; Daley M; Stalker D M
Calgene Inc., Davis, CA 95616.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jul 19 **1994**, 91 (15) p7301-5, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Phage **T7 RNA polymerase** has been used extensively in Escherichia coli for high-level expression of selected genes placed under the control of the phage **T7** gene 10 promoter. We have constructed an **analogous** system for use in plastids of higher plants. A **T7 RNA polymerase** chimeric gene containing a cauliflower mosaic virus 35S promoter and a tobacco ribulose-bisphosphate carboxylase/oxygenase small-subunit chloroplast transit-peptide sequence was introduced into tobacco by nuclear transformation. Stable plastid transformation of tobacco expressing the **T7 RNA polymerase** activity with a **T7** promoter/beta-glucuronidase (GUS) reporter gene construct resulted in expression of GUS mRNA and enzyme activity in all tissues examined. Expression of GUS activity was extremely high in mature leaves, moderate in young leaves and petals, and low in stems, roots, and developing seeds. Plastid transformation of wild-type tobacco with the same chimeric GUS gene resulted in undetectable levels of GUS mRNA and enzyme activity. Genetic crosses demonstrated that a silent **T7** /GUS reporter gene could be activated in the F1 generation by transmission of an active nuclear **T7 RNA polymerase** gene from the male parent.

5/3,AB/62 (Item 62 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08159335 94278492 PMID: 7516580
Structures of ternary complexes of rat DNA **polymerase** beta, a DNA template-primer, and ddCTP.
Pelletier H; Sawaya M R; Kumar A; Wilson S H; Kraut J
Department of Chemistry, University of California, San Diego 92093-0317.
Science (UNITED STATES) Jun 24 **1994**, 264 (5167) p1891-903, ISSN 0036-8075 Journal Code: 0404511
Contract/Grant No.: CA17374; CA; NCI; ES06839; ES; NIEHS; GM10928; GM; NIGMS
Comment in Science. 1994 Dec 23;266(5193) 2022-5
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Two ternary complexes of rat DNA **polymerase** beta (pol beta), a DNA template-primer, and dideoxycytidine triphosphate (ddCTP) have been determined at 2.9 A and 3.6 A resolution, respectively. ddCTP is the

triphosphate of dideoxycytidine (ddC), a nucleoside **analog** that targets the reverse transcriptase of human immunodeficiency virus (HIV) and is at present used to treat AIDS. Although crystals of the two complexes belong to different space groups, the structures are similar, suggesting that the **polymerase** -DNA-ddCTP interactions are not affected by crystal packing forces. In the pol beta active site, the attacking 3'-OH of the elongating primer, the ddCTP phosphates, and two Mg2+ ions are all clustered around Asp190, Asp192, and Asp256. Two of these residues, Asp190 and Asp256, are present in the amino acid sequences of all polymerases so far studied and are also spatially similar in the four polymerases--the Klenow fragment of Escherichia coli DNA **polymerase** I, HIV-1 reverse transcriptase, **T7 RNA polymerase** , and rat DNA pol beta--whose crystal structures are now known. A two-metal ion mechanism is described for the nucleotidyl transfer reaction and may apply to all polymerases. In the ternary complex structures analyzed, pol beta binds to the DNA template-primer in a different manner from that recently proposed for other **polymerase**-DNA models.

5/3,AB/63 (Item 63 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08085817 94233745 PMID: 8178462
Analysis of respiratory syncytial virus F, G, and SH proteins in cell fusion.
Heminway B R; Yu Y; Tanaka Y; Perrine K G; Gustafson E; Bernstein J M; Galinski M S
Department of Molecular Biology, Cleveland Clinic Foundation, Ohio 44195.
Virology (UNITED STATES) May 1 **1994**, 200 (2) p801-5, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Recombinant expression of the human respiratory syncytial virus (RSV) fusion (F) glycoprotein, receptor-binding glycoprotein (G), and small hydrophobic (SH) protein was performed to determine the role(s) of these proteins in syncytia formation. These studies used a vaccinia virus expressing the bacteriophage (**T7**) **RNA polymerase** gene and plasmid vectors containing the RSV genes under the control of a **T7** promoter. Within the context of this expression system, expression of any individual RSV gene, or coexpression of F+G genes, did not elicit the formation of syncytia. However, at plasmid input levels which were 10-fold higher than those normally used, coexpression of F+G induced low but detectable levels of cell fusion. In contrast, coexpression of F, G, and SH together elicited extensive cell fusion resembling that of an authentically infected cell monolayer. In addition, coexpression of F and SH elicited significant cell fusion, although to a lesser extent than was observed when G was included. Cell fusion induced by coexpression of F+SH was found to be specific to the RSV proteins, since coexpression of SH with the **analogous** F proteins from human parainfluenza virus type 3, human parainfluenza virus type 2, Sendai virus, or simian virus type 5 (SV5) did not elicit cell fusion. Finally, coexpression of the SV5 SH protein with the RSV or SV5 glycoproteins also failed to induce syncytia, suggesting type-specific restrictions between the two sets of viral proteins.

5/3,AB/64 (Item 64 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08025794 94148772 PMID: 8106324
Cloning and nucleotide sequence of the pvdA gene encoding the pyoverdin biosynthetic enzyme L-ornithine N5-oxygenase in Pseudomonas aeruginosa.
Visca P; Ciervo A; Orsi N

Istituto di Microbiologia, Universita di Roma La Sapienza, Italy.
Journal of bacteriology (UNITED STATES) Feb **1994**, 176 (4) p1128-40, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The enzyme L-ornithine N5-oxygenase catalyzes the hydroxylation of L-ornithine (L-Orn), which represents an early step in the biosynthesis of the peptidic moiety of the fluorescent siderophore pyoverdin in Pseudomonas aeruginosa. A gene bank of DNA from P. aeruginosa PAO1 (ATCC 15692) was constructed in the broad-host-range cosmid pLAFR3 and mobilized into the L-Orn N5-oxygenase-defective (pvdA) P. aeruginosa mutant PALS124. Screening for fluorescent transconjugants made it possible to identify the trans-complementing cosmid pPV4, which was able to restore pyoverdin synthesis and L-Orn N5-oxygenase activity in the pvdA mutant PALS124. The 17-kb PAO1 DNA insert of pPV4 contained at least two genetic determinants involved in pyoverdin synthesis, i.e., pvdA and pvdC4, as shown by complementation analysis of a set of mutants blocked in different steps of the pyoverdin biosynthetic pathway. Deletion analysis, subcloning, and transposon mutagenesis enabled us to locate the pvdA gene in a minimum DNA fragment of 1.7 kb flanked by two SphI restriction sites. Complementation of the pvdA mutation was under stringent iron control; both pyoverdin synthesis and L-Orn N5-oxygenase activity were undetectable in cells of the trans-complemented mutant which had been grown in the presence of 100 microM FeCl3. The entire nucleotide sequence of the pvdA gene, from which the primary structure of the encoded polypeptide was deduced, was determined. The pvdA structural gene is 1,278 bp; the cloned DNA fragment contains at the 5' end of the gene a putative ribosome-binding site but apparently lacks known promoterlike sequences. The P. aeruginosa L-Orn N5-oxygenase gene codes for a 426-amino-acid peptide with a predicted molecular mass of 47.7 kDa and an isoelectric point of 8.1. The enzyme shows approximately 50% homology with functional **analogs**, i.e., L-lysine N6-hydroxylase of aerobactin-producing Escherichia coli and L-Orn N5-oxygenase of ferrichrome-producing Ustilago maydis. The pvdA gene was expressed in P. aeruginosa under the control of the T7 promoter. Induction of the **T7 RNA polymerase** system resulted in parallel increases of the L-Orn N5-oxygenase activity and of the amount of a 47.7-kDa polypeptide. We also constructed a site-specific pvdA mutant by insertion of a tetracycline-resistance cassette in the chromosomal pvdA gene of P. aeruginosa PAO1.(ABSTRACT TRUNCATED AT 400 WORDS)

5/3,AB/65 (Item 65 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08001064 94118419 PMID: 8289375
Rescue of synthetic genomic **RNA analogs** of rabies virus by plasmid-encoded proteins.
Conzelmann K K; Schnell M
Federal Research Centre for Virus Diseases of Animals, Tubingen, Germany.
Journal of virology (UNITED STATES) Feb **1994**, 68 (2) p713-9, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Proteins entirely expressed from cDNA were used to rescue synthetic **RNA** genome **analogs** into infectious defective particles of rabies virus (RV). Synthetic negative-stranded RNAs containing 3'- and 5'-terminal RV sequences and transcriptional signal sequences were transcribed from plasmids transfected into cells expressing **T7 RNA polymerase** from recombinant vaccinia virus. After simultaneous expression of RV N, P, and L proteins from plasmids containing

a **T7 RNA polymerase** promoter, the synthetic genomes were encapsidated, replicated, and transcribed by the RV **polymerase** proteins. Insertion of the bacterial chloramphenicol acetyltransferase gene or beta-galactosidase (lacZ) gene between the 3' and 5' termini containing transcriptional signal sequences resulted in transcription of mRNAs and expression of chloramphenicol acetyltransferase and beta-galactosidase, respectively. Upon simultaneous expression of N, P, M, G, and L proteins, virions carrying the foreign genes were assembled and released into the supernatant. The possibility of rescuing cDNA into rabies virions by proteins also expressed entirely from cDNA opens the possibility of studying the functions of each RV protein and analyzing cis-acting signals of the RV genome.

5/3,AB/66 (Item 66 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07958467 94094074 PMID: 8269276
Construction of overlapping oligonucleotide templates for the production of cRNA standards for quantitative reverse transcription **polymerase** chain reaction.
Raftery K; Sharefkin J; Limanni A; Salomon R N
Department of Surgery, New England Medical Center Hospitals/Tufts University School of Medicine, Boston, MA 02111.
Diagnostic molecular pathology : the American journal of surgical pathology, part B (UNITED STATES) Jun **1993**, 2 (2) p120-4, ISSN 1052-9551 Journal Code: 9204924
Contract/Grant No.: RO4-HL-40680; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The reverse transcriptase **polymerase** chain reaction (PCR) is a technique for the study of gene expression that requires far less **RNA** for analysis than Northern blots. The inclusion of cRNA standards in the initial reverse transcription step is a way to control for the tube-to-tube variation often inherent in the technique and to permit quantitation of the starting amount of the native mRNA being analyzed. We describe a method using overlapping oligonucleotides to produce templates for the production of cRNA standards for up to three different mRNA species. The first step is the synthesis of a pair of overlapping oligonucleotides each of which encodes, respectively, sequences **analogous** to either sense or antisense primers for the PCR amplification of up to three different messages. These oligonucleotides are designed to have complementary 3' ends which permit spontaneous annealing and allow subsequent mutually priming extension of the annealed double-stranded portion by **T7** DNA **polymerase**. The **T7** and SP6 **RNA polymerase** promoters are then added to the ends of the template using standard PCR techniques. Once the template is assembled, **T7** and SP6 **RNA** polymerases are used to produce copious quantities of cRNA standards and controls. This technique can be used to construct multiple cRNA standards for essentially any messages of interest. Production of cRNA by a single **T7 RNA polymerase** reaction yields standards sufficient for several thousand separate reverse transcriptions.

5/3,AB/67 (Item 67 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07936766 94072578 PMID: 8251501
Mapping the central fold of tRNA2(fMet) in the P site of the Escherichia coli ribosome.
Rosen K V; Alexander R W; Wower J; Zimmermann R A
Department of Biochemistry and Molecular Biology, University of

Massachusetts, Amherst 01003.
Biochemistry (UNITED STATES) Nov 30 **1993**, 32 (47) p12802-11,
ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM22807; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

4-Thiouridine (s4U), a photoreactive **analog** of uridine, was randomly incorporated into tRNA2(fMet) precursor molecules by transcription with **T7 RNA polymerase**. The s4U-containing transcripts were trimmed at their 5'-ends with RNase P **RNA** to yield mature tRNA2(fMet). The photoreactive tRNA2(fMet) derivatives were aminoacylated and bound to the P site of 70S ribosomes from Escherichia coli in the presence of a poly(A,G,U) template. Irradiation of the complexes at 300 nm resulted in the covalent cross-linking of tRNA2(fMet) to ribosomal proteins and rRNAs within both the 50S and 30S subunits. The labeled proteins were identified as L1, L27, and S19. 50S-subunit proteins L1 and L27 were attached to nucleotide U17 or U17.1 within the D loop of tRNA2(fMet), whereas 30S-subunit protein S19 was cross-linked to nucleotide U47 in the variable loop. Both of these sites occur in or near the central fold of the tRNA. These results permit us to map the D loop of P site-bound tRNA to the region between the central protuberance and the L1 ridge on the 50S ribosomal subunit, while the variable loop can be placed above the cleft on the head of the 30S subunit.

5/3,AB/68 (Item 68 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07887514 94019469 PMID: 7692268
Mechanism of toxicity of 3-methyladenine for bacteriophage **T7**.
Racine J F; Zhu Y; Mamet-Bratley M D
Departement de Biochimie, Universite de Montreal, Que., Canada.
Mutation research (NETHERLANDS) Oct **1993**, 294 (3) p285-98,
ISSN 0027-5107 Journal Code: 0400763
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Treatment of bacteriophage **T7** with methyl methanesulfonate perturbed phage-specific genetic expression in both repair-proficient and repair-deficient Escherichia coli cells. In wild-type cells (AB1157), the time course of protein synthesis was slowed down but an entire complement of phage proteins was synthesized. In cells (BK2114, tag-) unable to repair 3-methyladenine, the toxic lesion produced by methyl methanesulfonate, alkylated phage produced only early (class I) proteins. These results suggested that late transcription was inhibited in infected tag- cells. These cells were shown to contain a significant amount of active **T7 RNA polymerase**, a class I protein. Thus, the cause of inhibition appeared to be the inability of **T7 RNA polymerase** to use unrepaired DNA as template. In vitro transcription assays with alkylated **T7** DNA as template supported this proposal. **T7 RNA polymerase** proved to be very sensitive to the presence of alkylation lesions. In addition, the phage enzyme was much more sensitive to these lesions than was its bacterial counterpart, E. coli **RNA polymerase**. These results suggest that 3-methyladenine exerts its toxic action, in the **T7** system, at the level of transcription by **T7 RNA polymerase**. To further characterize the reduced activity of the **T7** enzyme, an in vitro transcription assay using linearized plasmid DNA with one **T7** promoter was devised. Gel electrophoresis revealed that only one transcript of well-defined length was synthesized by **T7 RNA polymerase** on this template. Alkylation of the template did not alter the size of the

transcript produced. Simultaneous measurement of chain initiation and chain elongation confirmed this result by showing that both steps were reduced to the same extent by alkylation of template DNA. Thus **T7 RNA polymerase** does not appear to be blocked by 3-methyladenine. Rather the lesion must hinder translocation of **T7 RNA polymerase** along the DNA template during chain elongation.

5/3,AB/69 (Item 69 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07853079 93385110 PMID: 8373774
Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione.
Ren X; Bjornstedt M; Shen B; Ericson M L; Holmgren A
Department of Biochemistry I, Medical Nobel Institute, Karolinska Institutet, Stockholm, Sweden.
Biochemistry (UNITED STATES) Sep 21 **1993**, 32 (37) p9701-8, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A human thioredoxin cDNA was modified to optimize Escherichia coli expression and subcloned into the plasmid pACA, a vector for **T7 RNA polymerase** -directed expression. The substitution of structural (noncatalytic) half-cystines in human thioredoxin (hTrx) was made by site-directed mutagenesis. The recombinant wild-type (wt) hTrx and its mutant C61S, C72S, and C61S/C72S were expressed and purified to homogeneity. Characterization of the wt and mutant hTrx was done with respect to redox activity with thioredoxin reductase (TR), tryptophan fluorescence, and effects of incubation with GS-Se-SG, which is believed to be the major metabolite of inorganic selenium compounds in mammalian tissues. The Km and kcat of wild-type hTrx for human placenta thioredoxin reductase (HP-TR) at pH 7.0 were 2.0 microM and 2800 min-1, respectively. The mutant proteins C61S, C72S, and C61S/C72S had Km and kcat values similar to those of the wt thioredoxin. Tryptophan fluorescence measurements showed that the wt and mutant proteins had similar stability to a denaturing agent. Incubation of fully reduced thioredoxin with 0.1 molar equivalent of GS-Se-SG resulted in continued oxidation of SH groups. After 3.5 h only 0.5 of initially 4.6 SH groups/thioredoxin remained. With the oxidized protein, a pronounced lag phase in thioredoxin reductase-dependent insulin disulfide reduction was present. Disulfide-linked dimers of the protein were present. The results clearly showed that noncatalytic cysteine residues in hTrx were oxidized accompanied by dimerization and inactivation. The activities of the mutant proteins C72S and C61S/C72S were unchanged after 3 h of incubation with GS-Se-SG. No dimer appeared of the C72S thioredoxin.(ABSTRACT TRUNCATED AT 250 WORDS)

5/3,AB/70 (Item 70 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07843889 93374843 PMID: 8366033
Phenotype conversion in Pseudomonas solanacearum due to spontaneous inactivation of PhcA, a putative LysR transcriptional regulator.
Brumbley S M; Carney B F; Denny T P
Department of Plant Pathology, University of Georgia, Athens 30602-7274.
Journal of bacteriology (UNITED STATES) Sep **1993**, 175 (17) p5477-87, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Phenotype conversion (PC) in Pseudomonas solanacearum is the coordinated change in production of extracellular polysaccharide and a variety of extracellular proteins, some of which contribute to virulence. Although PC is normally spontaneous, it is mimicked by transposon inactivation of the phcA locus (S. M. Brumbley and T. P. Denny, J. Bacteriol. 172:5677-5685, 1990). The DNA sequence of a 1.8-kb region from strain AW1 that contains phcA revealed one open reading frame that should encode a polypeptide of 38.6 kDa. The PhcA protein produced in Escherichia coli by using a **T7 RNA polymerase** expression system was of the predicted size.
The deduced amino acid sequence of PhcA is similar to that of some members of the LysR transcriptional activator gene family, especially in the amino terminus, where a putative helix-turn-helix DNA-binding motif was identified. An **analogous** allele (phcA1) was cloned from the spontaneous PC mutant strain AW1-PC and found to be nonfunctional in complementation studies. When phcA1 was expressed in E. coli, the PhcA1 protein was 35.5 kDa, 3 kDa smaller than PhcA. Sequence analysis of phcA1 and chimeric constructs of phcA and phcA1 confirmed that PhcA1 is truncated by a 2-bp insertion 147 nucleotides upstream of the carboxyl terminus of PhcA. Southern blot analysis of 10 additional independently isolated PC mutants of strain AW1 revealed that two strains have larger insertions (0.2 and 1.0 kb) within phcA. These results suggest that phcA encodes a DNA-binding protein that regulates the transcription of one or more of the genes involved in P. solanacearum virulence and that spontaneous PC can be attributed to one of several different insertions within this locus.

5/3,AB/71 (Item 71 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07832822 93362426 PMID: 8395122
Rescue of synthetic **analogs** of genome **RNA** of human parainfluenza virus type 3.
De B P; Banerjee A K
Department of Molecular Biology, Research Institute, Cleveland Clinic Foundation, Ohio 44195.
Virology (UNITED STATES) Sep **1993**, 196 (1) p344-8, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: AI 32027; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A simple system that allows expression and packaging of a foreign gene by human parainfluenza virus type 3 (HPIV-3) has been described. First, a cDNA was constructed to encode an internally deleted version of HPIV-3 genome **RNA** . The viral genes were replaced with a negative sense copy of the bacterial chloramphenicol acetyl transferase (CAT) reporter gene. In vitro run-off transcription with **T7 RNA polymerase** synthesized an 870 nucleotide **RNA** that contained the antisense coding region of the CAT gene flanked by the transcription regulatory sequences and the 3' and 5' end extracistronic sequences of the HPIV-3 genome. When introduced into cells that are infected with HPIV-3, this **RNA** was amplified and the reporter gene was expressed, as measured by the CAT activity in the cell extract. Furthermore, the synthetic **RNA** was packaged into infectious virions. The addition of two extra nucleotides at the 5' end of the parental trailer region decreased the CAT activity by more than 90%, suggesting a requirement for the intact 5'-regulatory domain in the viral replicative cycle. Interestingly, the addition of one extra nucleotide to the 3' end totally abolished the CAT activity indicating that an exact 3' terminus is critical in this process.

5/3,AB/72 (Item 72 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07799176 93312541 PMID: 7763730
Cloning, expression, and characterization of a synthetic **analog** to the bioadhesive precursor protein of the sea mussel Mytilus edulis.
Salerno A J; Goldberg I
Biotechnology Department, Allied Signal Inc., Morristown, NJ 07962.
Applied microbiology and biotechnology (GERMANY) May **1993**, 39 (2) p221-6, ISSN 0175-7598 Journal Code: 8406612
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Repetitious gene cassettes that encode the consensus decapeptide repeat of Mytilus edulis bioadhesive protein were designed, constructed, and expressed in Escherichia coli. The bioadhesive precursor (BP) with a relative molecular mass of 25,000 was expressed from one 600-bp gene at levels approaching 60% of total cell protein in strains employing **T7 RNA polymerase** for induction and carrying a repetitious gene comprised of a 30-bp unit repeat that accounts for E. coli codon bias. BP forms intracellular inclusions and yet methionine was processed from the N-terminus of the purified protein, as shown by amino acid composition and N-terminal sequencing, to give an authentic consensus precursor protein.

5/3,AB/73 (Item 73 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07795963 93323109 PMID: 8331654
Bacteriophage **T7 RNA polymerase**. 19F-nuclear magnetic resonance observations at 5-fluorouracil-substituted promoter DNA and **RNA** transcript.
Rastinejad F; Lu P
Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia 19104.
Journal of molecular biology (ENGLAND) Jul 5 **1993**, 232 (1) p105-22, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have substituted 5-fluorodeoxyuridine (5-FdU) in place of thymidine in defined positions along synthetic bacteriophage **T7** promoter DNA sequences. None of the fluoro-substitutions in the promoter DNA sequence reduced transcription yields with **T7 RNA polymerase** significantly. Substitutions on the coding template strand reduced transcription yields when placed at +3, but not at +4. 19F-n.m.r. spectra from transcription reactions and gel analysis of transcription products show that **T7 RNA polymerase** correctly and efficiently utilizes 5-FUTP as a **RNA** substrate **analog**. The fluorine atom provides a sensitive probe for monitoring the local environment, base sequence and solvent exposure at the DNA major groove through its 19F-n.m.r. resonance. Buffer dependencies of the fluorine chemical shift and digestion patterns with DNase I suggest that the **T7** promoter base-pairs near the transcription start site are distorted with a more open minor groove and less solvent accessible major groove. Previous chemical footprinting data of promoter-**polymerase** complexes yield a picture that **T7 RNA polymerase** recognizes major groove features in the region from positions -7 to -11 and minor groove features on the same side of DNA flanking both sides of this region. Consistent with this, 19F-n.m.r. observations identify two additional positions, -8 and -17, involved in promoter recognition on this side of the DNA helix. On the other hand, our observations also implicate the opposite side of the DNA helix, primarily at positions -14 and -15, as major groove recognition

RNA polymerases.
Hanna M M; Zhang Y; Reidling J C; Thomas M J; Jou J
Department of Botany and Microbiology, University of Oklahoma, Norman 73019.
Nucleic acids research (ENGLAND) May 11 **1993**, 21 (9) p2073-9, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A new photocrosslinking CTP **analog** that functioned as a substrate during transcription was synthesized and used to photoaffinity label E. coli and bacteriophage **T7 RNA** polymerases. This **analog**, 5-((4-azidophenacyl)thio) cytidine-5'-triphosphate (5-APAS-CTP) contains an aryl azide group approximately 10 A from the nucleotide base and specifically replaced CTP during synthesis of **RNA** by both polymerases. **Analog** was placed at the 3' end or internally within **RNA** . Both polymerases inefficiently incorporated two 5-APAS-CMP molecules sequentially, as was found for the related 5-APAS-UMP. **Analog** was placed at the 3' end of **RNA** in transcription complexes paused at the site of Q-modification of E. coli **RNA polymerase**, downstream of the lambda PR' promoter (+16), a pause that requires specific DNA sequences but no apparent **RNA** hairpin. Crosslinking was examined in the presence and absence of the NusA protein, which enhances the transcriptional pause at this site and is required for Q modification of the **polymerase**. Crosslinking of the 3' end of the **RNA** to NusA was not observed, consistent with our earlier results involving a NusA-enhanced pause site downstream from an **RNA** hairpin.

5/3,AB/76 (Item 76 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07706439 93233245 PMID: 8386276
Rescue of synthetic **analogs** of genomic **RNA** and replicative-intermediate **RNA** of human parainfluenza virus type 3.
Dimock K; Collins P L
Laboratory of Infectious Disease, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.
Journal of virology (UNITED STATES) May **1993**, 67 (5) p2772-8, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The genome of human parainfluenza virus type 3 (PIV3) is a single negative-sense **RNA** strand (vRNA) that is 15,463 nucleotides in length. A cDNA was constructed to encode an 898-nucleotide, internally deleted version of PIV3 vRNA, PIV3-CAT vRNA, in which the viral genes were replaced with the bacterial chloramphenicol acetyltransferase (CAT) reporter gene. The CAT gene was flanked in turn by sequences representing (i) nontranslated sequences of the first and last genes in the PIV3 genome, (ii) PIV3 gene-start and gene-end sequences, which are presumed to be transcription signals, and (iii) 3' extracistronic (leader) and 5' extracistronic (trailer) terminal regions of PIV3 vRNA. A second cDNA was constructed to encode the exact complement of PIV3-CAT vRNA; this positive-sense **RNA**, PIV3-CAT vcRNA, would correspond to the predicted replicative intermediate of PIV3-CAT vRNA. When synthesized in vitro by runoff transcription with **T7 RNA polymerase** and transfected separately into PIV3-infected cells, both PIV3-CAT vRNA and vcRNA were rescued with similar efficiencies; that is, they were expressed to yield CAT and were packaged into particles that could be used to infect fresh cells. Rescue of PIV3-CAT vRNA was strictly dependent on complementation by PIV3; PIV3 could not be replaced by respiratory

sites for **T7 RNA polymerase**. In addition, n.m.r. spectra from 5-FdU-substituted base-pairs -2 and -3, suggest either additional interactions on the same side of the DNA helix as -14 and -15, or distortions in the DNA structure.

5/3,AB/74 (Item 74 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07753453 93277830 PMID: 8504073
Laser-induced protein-DNA cross-links via psoralen furanside monoadducts.
Sastry S S; Spielmann H P; Hoang Q S; Phillips A M; Sancar A; Hearst J E
Department of Chemistry, University of California, Berkeley.
Biochemistry (UNITED STATES) Jun 1 **1993**, 32 (21) p5526-38,
ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM 32833; GM; NIGMS; GM41911; GM; NIGMS; NIEHS 07075-11; EH; NCEH
Erratum in Biochemistry 1994 Feb 15;33(6) 1616
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have developed a technique for cross-linking DNA binding proteins to DNA using psoralen furanside monoadducts as photoaffinity probes and a continuous-wave argon ion laser (366 nm) as a light source. Several DNA binding proteins (**T7 RNA polymerase**, UvrB, single-stranded DNA binding protein of Escherichia coli, T4 gp32, and RecA of E. coli) are shown to cross-link to single-stranded psoralen monoadducted DNA oligos differing in length and sequence. Increasing fluences of laser light on a fixed ratio of DNA/protein resulted in an increase in the yield of cross-links. Titration experiments were carried out to measure the apparent cross-linking constant (KappXL) for **T7 RNA polymerase** or UvrB to a monoadducted 24 mer DNA. The estimated values for the apparent cross-linking constant were in the range of (2-3) x 10(-7) M for both **T7 RNA polymerase** and UvrB. The efficiency of cross-linking was investigated as a function of the length of adducted DNA and also as a fraction of the total noncovalent binding of proteins of psoralenated DNAs. The results showed that in the cases of **T7 RNA polymerase** and UvrB cross-linking was more efficient with short oligos (8 and 19 mers) as compared to longer oligos (50 mer). A tryptic peptide of **T7 RNA polymerase** that was conjugated to a psoralen furanside monoadducted 12 mer DNA was isolated by high-performance liquid chromatography. Mass spectrometry and amino acid composition of this peptide revealed that it originated from a region between residues 558 and 608 of the primary structure of **T7 RNA polymerase**. Two other peptides cross-linked to oligos were also purified. Repeated attempts to perform Edman sequencing of the peptide-DNA conjugates failed. Overall evidence indicates that photo-cross-linking of furanside monoadducts occurred at multiple sites on the proteins. We have shown that **T7 RNA polymerase** molecules in a ternary complex arrested at the furanside monoadduct can be cross-linked to the DNA templates with laser light. Evidence suggests that the arrested **polymerase** molecules existed in multiple conformations on the DNA template. This method of transcriptional cross-linking offers a new method for preparing highly stable elongation complexes for further studies.

5/3,AB/75 (Item 75 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07751721 93275732 PMID: 7684833
Synthesis and characterization of a new photocrosslinking CTP **analog** and its use in photoaffinity labeling E. coli and **T7**

5/3,AB/59 (Item 59 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08306090 95065720 PMID: 7975272
Reverse misreading of a GC doublet by the modified **T7** DNA **polymerase**, Sequenase.
Odagiri T
Department of Virology, Jichi Medical School, Tochigi, Japan.
Virus genes (UNITED STATES) Jul **1994**, 8 (3) p271-4, ISSN 0920-8569 Journal Code: 8803967
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In nucleotide sequencing of the cDNA of the influenza virus PB2 **polymerase** gene by the dideoxy method using a modified **T7** DNA **polymerase** , Sequenase, the sequence of the promoter region, 5'-AGCGAAAGCAGG, was shown to be misread as 5'-AGCGAAACGAGG, i.e., a GC doublet at positions 8 and 9 was read in reverse. This misreading was also found both when the sequence of BsmI restriction site upstream from the PB2 promoter sequence was exchanged by that of the promoter of **T7** **RNA polymerase** and when the downstream region was substituted with the nonstructural (NS) protein gene. These results indicated that the misreading by Sequenase was attributed specifically to the PB2 promoter region, independent of the upstream and downstream sequences. The misreading, however, did not occur when dGTP in the labeling mixture was substituted with another nucleotide **analog**, dITP. Furthermore, the reversion did not occur in the NS gene promoter region, where the nucleotide sequence was 5'-AGCAAAAGCAGG. Since the nucleotide difference between the PB2 and NS promoter regions was only at the fourth residue, i.e., G for PB2 and A for PB2 and A for NS, the G residue followed by a triplet AAA in the PB2 promoter region was suggested to be a signal responsible for the misreading by Sequenase **T7 DNA polymerase**. The findings warns of possible misreading in determining DNA sequences, in addition to compression of the sequencing ladder.

5/3,AB/60 (Item 60 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08208287 94344242 PMID: 8065453
Interactions of a small **RNA** with antibiotic and **RNA** ligands of the 30S subunit.
Purohit P; Stern S
Program in Molecular Medicine, UMASS Medical Center, Worcester 01605.
Nature (ENGLAND) Aug 25 **1994**, 370 (6491) p659-62, ISSN 0028-0836 Journal Code: 0410462
Comment in Nature. 1994 Aug 25;370(6491) 597-8; Comment in PMID 8065445
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It is now generally accepted that 16S and 23S ribosomal **RNA** play important roles in the decoding and peptidyl transferase activities of ribosomes. Despite their complex structures and numerous associated proteins it is possible that small domains of these rRNAs can fold and function autonomously, particularly those that appear devoid of protein interactions. One candidate for such a domain is the decoding region, located near the 3' end of 16S rRNA (Fig. 1a, b). Consistent with this hypothesis, aminoglycoside antibiotics that interact with the decoding region in 30S subunits interact with other RNAs in the absence of proteins. In addition, certain activities of self-splicing introns, at least

IL, 60637: jpicciri@midway.uchicago.edu, jschwans@midway.uchicago.edu**
USA
JOURNAL: Abstracts of Papers American Chemical Society 222 (1-2):pBIOL162
2001
MEDIUM: print
CONFERENCE/MEETING: 222nd National Meeting of the American Chemical Society
Chicago, Illinois, USA August 26-30, 2001
SPONSOR: American Chemical Society
ISSN: 0065-7727
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The unique physiochemical properties of sulfur render it one of the most powerful tools for exploring the structure and function of nucleic acids. To exploit these properties in probing the role of the 2'-hydroxyl group in **RNA** mediated processes, we have synthesized a series of 2'-mercapto-5'-O-(1-thio)-nucleoside triphosphates for use in nucleotide analogue interference mapping (NAIM) experiments. The 2'-deoxy-2'-t-butyl disulfide protected nucleosides of cytidine, uridine, adenosine, and **inosine** were synthesized and converted to their alpha-thiotriphosphates in a one-pot, two step reaction. Reduction of the disulfide by DTT provided the alpha-thiotriphosphates. The analogues are substrates for the Y639F mutant **T7 RNA polymerase**, providing the potential to replace every 2'-hydroxyl group with a mercapto group in an individual **RNA**. Using the Tetrahymena ribozyme as a model system we incorporated these nucleoside derivatives randomly by in vitro transcription. NAIM experiments have revealed sites of interference that in some cases are distinct from those arising from 2'-deoxy and 2'-methoxy substitution. We are currently investigating the molecular basis of this interference.

2001

```
 b 155, 5
      29dec02 14:36:12 User242957 Session D563.2
           $0.00    0.071 DialUnits File410
     $0.00  Estimated cost File410
     $0.05  TELNET
     $0.05  Estimated cost this search
     $0.05  Estimated total session cost   0.220 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Nov W4
*File 155: For updating information please see Help News155. Alert
feature enhanced with customized scheduling. See HELP ALERT.
  File   5:Biosis Previews(R)  1969-2002/Dec W4
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? s inosine and rna and polymerase and t7
          11627  INOSINE
         714059  RNA
         374087  POLYMERASE
          11935  T7
      S1      7  INOSINE AND RNA AND POLYMERASE AND T7
? rd
...completed examining records
  -   S2      5  RD (unique items)
? t s2/3,ab/all

 2/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10441073   99436120   PMID: 10506170
   Bipartite modular structure of intrinsic, RNA hairpin-independent
termination signal for phage RNA polymerases.
  Kwon Y S; Kang C
  Department of Biological Sciences, Korea Advanced Institute of Science
and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea.
  Journal of biological chemistry (UNITED STATES)  Oct 8 1999,  274  (41)
 p29149-55,  ISSN 0021-9258  Journal Code: 2985121R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The phage SP6 RNA and T7 RNA polymerases, which are
closely related to each other, intrinsically stop at two signals in the
Escherichia coli rrnB terminator t1 through different mechanisms. The
downstream signal functioned without an RNA secondary structure
formation, in which the signal was still active when separated from the
upstream, hairpin-forming signal, and IMP incorporation enhanced its
efficiency. The sequence from -15 to -1 was essential for the downstream,
hairpin-independent termination (at -1). The results of SP6 transcription
with heteroduplex templates and ribonucleotide analogs suggested that the
downstream signal consists of two functionally different modules. The
effects of iodo-CMP or IMP incorporation into RNA on termination
efficiency were not sensitive to incorporation at -9 and upstream, but they
were reactive to incorporation at -6 and -2, as reflected by strong
iodo-rC:dG and weak rI:dC base pairing. Thus, the downstream module (from
-8 approximately -6 to -1) appears to facilitate the release of RNA.
Mismatches in the templates at -6 to +1 allowed for efficient termination,
unlike those upstream of the sequence. The upstream module (from -15 to -9
approximately -7) functions as a duplex. Pausing of the SP6 elongation
complex at the termination site was detected when RNA release was
```

suppressed by the incorporation of 5-bromo-UMP, and it was dependent on the upstream module. Results of single-round SP6 transcriptions using 3'-deoxynucleotides and immobilized templates indicated that **RNA** was not released from the elongation complexes halted at the termination site on the template variants carrying mutations in the upstream or downstream module, whereas such complexes on the wild type template were dissociated. Thus, halting or simple pausing was not sufficient for termination even when the downstream module was intact. The upstream module appears to mediate such conformation change necessary for termination.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09979611 98409449 PMID: 9737873
Identifying **RNA** minor groove tertiary contacts by nucleotide analogue interference mapping with N2-methylguanosine.
Ortoleva-Donnelly L; Kronman M; Strobel S A
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
Biochemistry (UNITED STATES) Sep 15 1998, 37 (37) p12933-42, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Nucleotide analogue interference mapping (NAIM) is a general biochemical method that rapidly identifies the chemical groups important for **RNA** function. In principle, NAIM can be extended to any nucleotide that can be incorporated into an in vitro transcript by an **RNA polymerase**. Here we report the synthesis of 5'-O-(1-thio)-N2-methylguanosine triphosphate (m2GalphaS) and its incorporation into two reverse splicing forms of the Tetrahymena group I intron using a mutant form of **T7 RNA polymerase** . This analogue replaces one proton of the N2 exocyclic amine with a methyl group, but is as stable as guanosine (G) for secondary structure formation. We have identified three sites of m2GalphaS interference within the Tetrahymena intron: G22, G212, and G303. All three of these guanosine residues are known to utilize their exocyclic amino groups to participate in tertiary hydrogen bonds within the ribozyme structure. Unlike the interference pattern with the phosphorothioate of **inosine** (IalphaS, an analogue that deletes the N2 amine of G), m2GalphaS substitution did not cause interference at positions attributable to secondary structural stability effects. Given that the **RNA** minor groove is likely to be widely used for helix packing, m2GalphaS provides an especially valuable reagent to identify **RNA** minor groove tertiary contacts in less well-characterized RNAs.

2/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05588031 88011325 PMID: 2821285
Mapping and characterization of transcriptional pause sites in the early genetic region of bacteriophage **T7**.
Levin J R; Chamberlin M J
Department of Biochemistry, University of California, Berkely 94720.
Journal of molecular biology (ENGLAND) Jul 5 1987, 196 (1) p61-84, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: GM12010; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
During transcription of DNA templates in vitro, Escherichia coli

RNA polymerase pauses at certain sequences before resuming elongation. Previous studies have established that some pausing events are brought about by the formation of **RNA** hairpin structures in the nascent transcript; however, it is not known whether this is an invariant and causal relationship. We have mapped and characterized almost 200 distinct pause sites located within the early region of bacteriophage **T7** DNA using a collection of **T7** deletion mutant DNAs and taking advantage of a procedure that permits synchronous transcription from the **T7** A1 promoter. The pausing pattern is sensitive both to the overall concentration of nucleotide substrates and to the relative concentrations of the four nucleotides. The apparent Ks value for a particular nucleoside triphosphate can vary over a 500-fold range depending on the nucleotide sequence, and pausing at some sites can be induced by modest reductions in substrate concentrations. However, pausing is not solely a consequence of substrate limitation. Pausing at certain sites is caused by some feature of the template or of the transcript itself. Substitution of **inosine** triphosphate (ITP) for GTP during transcription strongly affects the pattern and strength of pausing events, suggesting that base-pairing interactions involving the **RNA** strand are important for some pausing events. Other pauses are determined by sequences downstream from the elongation site that have not yet been transcribed, and pausing at these sites is generally insensitive to substitution of IMP for GMP in the nascent transcript. Pausing at one particular site on **T7** DNA is strongly enhanced by the presence of E. coli gene nusA protein. These results confirm that there are multiple classes of sites that lead to transcriptional pausing, and provide a collection of sites for further study. Using selected pause sites in the early region of **T7** DNA, we have tried to evaluate the possible roles of primary sequence, base composition and secondary structure in pausing. Computer analysis was used to compare primary sequences and potential **RNA** hairpin structures in transcripts for pauses known to share similar biochemical properties. We see no correlation of pause sites with regions of particular base composition or with specific primary sequences. While some pauses are correlated with the potential to form stable **RNA** hairpins just upstream from the growing point of the **RNA** chain, there is not a strict one-to-one relationship between predicted **RNA** hairpins and the location of pause sites.(ABSTRACT TRUNCATED AT 400 WORDS)

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01594272 73134630 PMID: 4144107
Characterization of **T7**-specific ribonucleic acid **polymerase**. II. Inhibitors of the enzyme and their application to the study of the enzymatic reaction.
Chamberlin M; Ring J
Journal of biological chemistry (UNITED STATES) Mar 25 1973, 248 (6) p2245-50, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

2/3,AB/5 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13306665 BIOSIS NO.: 200100513814
2'-Mercaptonucleosides as **RNA** structure/function probes.
AUTHOR: Piccirilli Joseph A(a); Schwans Jason P(a); Cortez Cecilia N(a)
AUTHOR ADDRESS: (a)Department of Biochemistry and Molecular Biology and Chemistry, HHMI, University of Chicago, 5841 S. Maryland Ave, Chicago,

syncytial virus or, unexpectedly, by a bovine strain of PIV3. Passage was blocked by prior incubation with neutralizing monoclonal antibodies specific to the PIV3 attachment protein. Also, during nine serial passages, the expression of CAT by PIV3-CAT vRNA increased more than 3,000-fold. These results indicated that the 3'-terminal 111 nucleotides and the 5'-terminal 115 nucleotides of PIV3 vRNA, which are present in PIV3-CAT vRNA, contained all of the cis-acting **RNA** sequences required for replication, gene expression, and transmission.

5/3,AB/77 (Item 77 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07704915 93231536 PMID: 8097177
Biogenesis and regulation of the Vibrio cholerae toxin-coregulated pilus: **analogies** to other virulence factor secretory systems.
Kaufman M R; Shaw C E; Jones I D; Taylor R K
Department of Microbiology and Immunology, University of Tennessee, Memphis 38163.
Gene (NETHERLANDS) Apr 15 **1993**, 126 (1) p43-9, ISSN 0378-1119 Journal Code: 7706761
Contract/Grant No.: AI-07238; AI; NIAID; AI-25096; AI; NIAID; DK077405-07 ; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Biogenesis of the toxin-coregulated pilus (TCP) of Vibrio cholerae 01 is essential for successful bacterial colonization of the small intestine. Pilus assembly requires the products of at least seven genes located on the chromosome adjacent to the pilin-encoding gene, tcpA. Previously reported TnphoA insertions in the TCP-assembly-deficient V. cholerae strains, KP2.21 and KP4.2, were isolated from the chromosome for further analysis. Nucleotide sequencing of the tcpE::phoA and tcpF::phoA fusions and corresponding clones of the region containing the intact genes revealed the presence of two open reading frames (ORFs) of 340 and 338 amino acids, designated TcpE and TcpF, respectively. The partial sequence of an ORF downstream from the TcpF coding sequence was determined to correspond to the global virulence regulator, ToxT. Proteins corresponding to the observed ORFs were visualized with the **T7** promoter/**RNA** **polymerase** expression system. Computer-generated alignment algorithms predict that a homology exists between TcpE and the Klebsiella pneumoniae pullulanase secretion proteins PulD and PulF, the Xanthomonas campestris extracellular enzyme secretion factor XpsF, the Bacillus subtilis DNA competence protein ComG-ORF2, and the Yersinia enterocolitica Yop secretion determinant YscC. These observations provide a model to investigate further the relationship between the secretion mechanisms utilized by these seemingly diverse virulence determinants. Additionally, an extreme C-terminal segment of TcpE shows striking homology to the transmembrane segment of the eukaryotic integrin beta-1 chain, which could imply a role for TcpE in not only TCP secretion, but also host cell interaction.

5/3,AB/78 (Item 78 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07703432 93229586 PMID: 8471665
Study of phage **T7** DNA-dependent **RNA polymerase** using GTP **analogs**. Affinity modification and study of interaction with matrices using fluorescent markers]
Issledovanie DNK-zavisimoi RNK-polimerazy faga **T7** s pomoshch'iu **analogov** GTP. Affinaia modifikatsiia fluorestsentnymi metkami i issledovanie vzaimodeistviia s matritsei.
Mishin A A; Khropov Iu V; Tunitskaia V L; Kochetkov S N